COMMUNITY EVOLUTION INCREASES ECOSYSTEM FUNCTIONING AND STABILITY

Dissertation

zur
Erlangung der naturwissenschaftlichen Doktorwürde
(Dr. sc. nat.)

vorgelegt der

Mathematisch-naturwissenschaftlichen Fakultät
der
Universität Zürich

von

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Zürich, 2017

I dedicate this thesis to my family.

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SUMMARY

Science is voiceless; it is the scientists who talk.

- Simone Weil (1942)

Biodiversity is important for many ecosystem functions, such as plant productivity and stability of ecological communities. Previously it was shown that the positive effect of biodiversity on plant primary productivity increases with time. Therefore, this thesis aimed to integrate evolutionary theory and experimental approaches into the community ecology approach of biodiversity experiments.

In the **first Chapter**, I discuss the influence of community evolution on the biodiversity–ecosystem functioning relationships in a large grassland biodiversity experiment, namely the Jena Experiment. I compared community productivity of selected plant communities, in which species had been co-selected for eight years, to naïve communities with identical species composition, but where the species were lacking this history of co-occurrence. I found that community evolution in selected plant communities increased community productivity at low diversity up to four species, but not at higher diversity levels. These results suggest that community evolution can lead to increased ecosystem functioning potentially via niche differentiation but only when a community is comprised of few species. I propose that conservation strategies should consider protecting species within a community context and not in isolation, in particular when it comes to communities already suffering from the loss of some of their constituents.

In the **second Chapter**, I used the same data as in Chapter 1 to establish whether community evolution also enhances community stability during unperturbed states and in response to an extreme weather event. In spring 2013, the experimental field site in Jena was flooded, which provided a unique opportunity to study the response of our co-selected and naïve plant communities to this extreme event. I found that selected plant communities recovered better from this event and that they were also more temporally stable before and after the event. These results further emphasize the implications of the findings of the first Chapter. Species need to be preserved in their community context to ensure ecosystem functioning and ecosystem stability, in particular in terms of resilience to environmental perturbations.

In the **third Chapter**, I report results from a glasshouse pot experiment conducted in Zurich. Here, I switched gears and analyzed the differentiation of selected plant populations within a species into monoculture and mixture types. I conducted an experiment comparing 2-species test mixtures consisting of plants with a background of growing in monoculture for twelve years with 2-species mixtures consisting of plants with a background in mixtures. I also studied test monocultures of four individuals either consisting of mixture-type plants and monoculture-type plants. I found that the past in either mixture or monoculture significantly affected plant performance in these newly assembled test communities, but my results depended on both species identity and species combination. I furthermore observed differences in plant traits, indicating that monoculture type plants increased their niche width during the course of the selection in monoculture. These findings suggest that community diversity can act as selective power differentiating populations within spies into mixture and monoculture sub-types. I propose that further studies need to be

conducted with more species and species combinations and that genetic analyses would be essential to find the underlying mechanism.

In the **fourth Chapter**, I present the results of a collaborative effort with a Dutch group. We sequenced plant material from the glasshouse experiment described in the third chapter and tested for epigenetic and genetic differentiation. Because we observed phenotypic trait differences between monoculture-type plants and mixture-type plants, we aimed to find out if genetic or epigenetic differences were driving these observations. I found that plants clustered according to single nucleotide polymorphisms (SNPs), which are tiny differences in the base-pairs of the DNA. Epigenetic differences followed the underlying genetic variation. I propose that selection on standing genetic variation at the beginning of the field experiment has led to the selection of different genotypes best equipped for their respective community diversity environment. These findings show that rapid evolution can happen in the field in perennial grassland species even in the absence of many generations of population growth.

In the **fifth Chapter**, we expanded the focus and included interactions of plants with their belowground partners, in particular arbuscular mycorrhizal fungi (AMF). In a glasshouse pot experiment conducted by Terhi Hahl, we compared plants selected in monocultures vs. plants selected in mixtures and grew them individually in either the presence of AMF from monoculture or from mixture plots. Furthermore, we included two control treatments, a negative control (sterilized soil) and a positive control (the widespread and well-known AMF species *Rhizoglomus irregulare*). We found mixed evidence for co-adaptation between AMF from mixtures and mixture-type plants and AMF from monoculture and monoculture-type plants. The outcome of the interaction depended on the species and plant functional group. Furthermore, associations with AMF were more often detrimental than beneficial to the plants. These results suggest that AMF and plant interactions are context-dependent, but that co-adaptation can occur within ecologically relevant time periods.

In summary, my results emphasize the importance of including evolutionary approaches in plant community ecology. Only such an integrative approach will result in a deeper mechanistic understanding of the positive effect of biodiversity on ecosystem functioning and ecosystem services crucial for human wellbeing.

ZUSAMMENFASSUNG

Wo der mystische Glaube anfängt, hört die echte Wissenschaft auf. Beide Tätigkeiten des menschlichen Geistes sind scharf voneinander zu halten.

- Ernst Haeckel in Natürliche Schöpfungsgeschichte (1898)

Biodiversität ist wichtig für viele Ökosystemfunktionen wie zum Beispiel die Pflanzenproduktivität oder die Stabilität von ökologischen Gemeinschaften. Es wurde gezeigt, dass dieser positive Effekt von Biodiversität auf die Pflanzenproduktivität mit der Zeit zunimmt. Darum war das Ziel dieser Dissertation, evolutionäre Theorie und Experimente in den für die Ökologie von Artengemeinschaften üblichen Ansatz zu integrieren.

Im **ersten Kapitel** diskutiere ich den Einfluss von gemeinschaftlicher Evolution auf den Zusammenhang zwischen Biodiversität und Ökosystemfunktionen in einem grossen Wiesenbiodiversitätsexperiment (Jena Experiment). Ich habe die Gemeinschaftsproduktivität von selektionierten Pflanzengemeinschaften, in welchen die verschiedenen Pflanzenarten über acht Jahre koselektioniert wurden, mit der Produktivität von naiven Gemeinschaften, die aus denselben Arten bestanden, aber keine solche gemeinsame Vergangenheit hatten, verglichen. Die gemeinschaftliche Evolution erhöhte die Pflanzenproduktivität, aber nur bis zu einer Diversität von vier Arten. Die gemeinschaftliche Evolution hatte keinen Einfluss in 8-Arten-Mischungen. Dies lässt vermuten, dass die gemeinschaftliche Evolution die Ökosystemfunktion, möglicherweise durch eine Differenzierung der Nischen, nur in Gemeinschaften mit wenigen Arten verbessern kann. Ich empfehle in Arterhaltungsstrategien zu berücksichtigen, dass man Arten in ihrer Gemeinschaft schützen sollte und nicht voneinander isoliert. Dies ist möglicherweise besonders wichtig für diejenigen Artengemeinschaften, welche schon am Verlust einiger ihrer Mitglieder leiden.

Im **zweiten Kapitel** nutze ich dieselben Daten wie im ersten Kapitel, um herauszufinden, ob die gemeinschaftliche Evolution zusätzlich auch die Stabilität während ungestörten Zeiten und als Reaktion auf eine Flut erhöhen kann. Im Frühling 2013 wurde unser Feldexperiment überflutet, was eine einmalige Möglichkeit bot, die Reaktion der naiven und koselektionierten Gemeinschaften auf dieses Extremereignis zu testen. In der Tat erholten sich die selektionierten Gemeinschaften besser von diesem Ereignis und waren auch über die Zeit stabiler. Diese Resultate verdeutlichen nochmals die Schlussfolgerungen aus dem ersten Kapitel. Arten müssen innerhalb ihrer Gemeinschaft geschützt werden, um die Ökosystemfunktionen und Ökosystemstabilität zu bewahren. Dies ist vor allem wichtig im Sinne der Widerstandfähigkeit gegenüber Umweltstörungen.

Im **dritten Kapitel** berichte ich von Resultaten von einem Gewächshausexperiment in Zürich. In diesem Kapitel verfolge ich einen anderen Ansatz und analysiere die Differenzierung von Pflanzenpopulationen innerhalb einer Art in Mischungstypen und Monokulturtypen. Ich habe ein Experiment durchgeführt, in dem ich 2-Arten-Mischungen bestehend aus Pflanzen mit einem Hintergrund in Mischungen und 2-Arten-Mischungen bestehend aus Pflanzen mit einer Vergangenheit in Monokulturen miteinander verglich. Zusätzlich habe ich auch Monokulturen bestehend aus entweder Pflanzen aus Mischungen oder Monokulturen angepflanzt. Die Vergangenheit entweder in Mischung oder Monokultur hatte einen signifikanten Einfluss darauf, wie sich die Pflanzen in diesen neuen Test-Gemeinschaften hinsichtlich ihrer Produktivität verhielten. Die Resultate waren aber

sehr von der Art oder Artkombination abhängig. Ich habe auch beobachtet, dass einzelne Pflanzenmerkmale sich unterschiedlich verändert hatten. Monokulturtypen schienen ihre Nische im Verlaufe der Zeit des Experiments verbreitert zu haben. Diese Resultate zeigen, dass Gemeinschaftsdiversität als Selektionsdruck funktionieren kann und dass dieser Druck Pflanzenpopulationen in Mischungs- und Monokulturtypen differenzieren kann. Ich schlage vor, dass weitere Experimente mit weiteren Arten und Artkombinationen durchgeführt werden und dass genetische Analysen von grosser Wichtigkeit sind, um die Mechanismen zu bestimmen.

Im **vierten Kapitel** präsentiere ich die Resultate einer Kollaboration mit einer holländischen Forschungsgruppe. Wir haben Pflanzenmaterial vom Gewächshausexperiment aus dem dritten Kapitel sequenziert und getestet, ob die im dritten Kapitel beschriebenen phänotypischen Beobachtungen eine genetische oder epigenetische Grundlage haben. Wir haben herausgefunden, dass die Pflanzenindividuen sich anhand von Einzelnukleotid-Polymorphismen (Variation in einem einzelnen Basenpaar in einem DNA-Strang) aufteilen. Epigenetische Unterschiede folgten der darunterliegenden genetischen Variation. Dies deutet darauf hin, dass die Selektion auf die genetische Variation zu Beginn des Experiments dazu führte, dass spezifisch die Genotypen, die an ihre jeweilige Gemeinschaftsdiversität gut angepasst waren, überlebten. Diese Resultate zeigen, dass schnelle Evolution im Feld vorkommen kann, auch bei mehrjährigen Pflanzen und in Abwesenheit von vielen Generationen von Populationswachstum.

Im **fünften Kapitel** habe ich den Fokus auf Interaktionen zwischen Pflanzen und den mit ihnen assoziierten Bodenorganismen, spezifisch arbuskuläre Mykorrhizapilze, erweitert. In einem von Terhi Hahl durchgeführten Gewächshausexperiment haben wir erneut Pflanzen mit einem Mischungshintergrund und Pflanzen mit einem Monokulturhintergrund miteinander verglichen und sie zu diesem Zweck einzeln in Töpfen wachsen lassen. In die Erde haben wir entweder Mykorrhizapilze aus Pflanzenmischungen oder Mykorrhizapilze aus Pflanzenmonokulturen hinzugefügt. Wir haben einige Hinweise dafür gefunden, dass die Pilze jeweils mit den Mischungstypen oder Monokulturtypen koadaptiert waren. Der Ausgang des Experiments hing aber stark von der betreffenden Pflanzenart oder funktionellen Gruppe ab. Ausserdem waren die Assoziationen der Pilze mit den Pflanzen häufiger nachteilig als – wie eigentlich erwartet – nutzbringend. Diese Resultate zeigen, dass Pflanzen-Pilz-Interaktionen sehr kontextabhängig sind und dass Koadaptation innerhalb von ökologisch relevanten Zeitspannen möglich ist.

Zusammenfassend verdeutlichen meine Resultate, dass es wichtig ist, evolutionäre Ansätze in die Ökologie von Artengemeinschaften mit einzubeziehen. Nur ein solch integrativer Ansatz wird zu einem besseren mechanistischen Verständnis der positiven Effekte von Biodiversität auf Ökosystemfunktionen und Ökosystemdienstleistungen führen.

INTRODUCTION

It may be argued, therefore, that the essential qualities which determine the ecology of a species may only be detected by studying the reaction of its individuals to their neighbours and that the behavior of individuals of the species in isolation maybe largely irrelevant to understanding their behaviour in the community.

-John L. Harper in The individual in the population (1964)

The biodiversity–ecosystem functioning (BEF) relationship

In 1959 Hutchinson concluded that "the reason why there are so many species of animals is at least partly because a complex trophic organization of a community is more stable than a simple one [...]" (Hutchinson 1959). At that time, little empirical evidence existed to support his notion, but his idea accompanied biodiversity–ecosystem functioning (BEF) research ever since. The biodiversity–ecosystem functioning relationship comprises two players, which I briefly want to introduce. The first player, biodiversity, describes genetic variation within species, species richness within communities as well as landscape heterogeneity within a region (Hooper *et al.* 2005). Consequently, biodiversity can be viewed in terms of numbers of entities (how many genotypes, species, or ecosystems), the evenness of their distribution and the differences in their functional traits (functional diversity). The second player, the functioning of an ecosystem, can be described by ecosystem properties such as productivity, carbon storage, hydrology and nutrient cycling (Hooper *et al.* 2005). In this thesis, the term biodiversity will be used for species richness (number of entities) within communities and ecosystem functioning will be represented by plant productivity, i.e. plant aboveground biomass produced over a given time period.

Since the time Hutchinson made his claim, a vast number of empirical and modeling studies supported his original idea and today there is a consensus in zoological, botanical and microbial research that biodiversity is crucial for the functioning of a variety of different ecosystems (Hooper *et al.* 2005). For example, in grasslands more diverse plant communities were shown to have a more stable productivity over time (Allan *et al.* 2011; Gross *et al.* 2014; Isbell *et al.* 2015), to be more productive (Tilman *et al.* 2001) or to be more resilient towards external perturbations (see Chapter 2). In forests, the positive influence of biodiversity on ecosystem functioning was furthermore shown in increased forest productivity (Liang *et al.* 2016), soil carbon sequestration, nutrient retention and pest resistance (Verheyen *et al.* 2016). Biodiversity facilitated resilience in freshwater ecosystems (Downing & Leibold 2010), and increased both stability and productivity in marine ecosystems. In microbiology, ecosystem functions such as respiration rate (Fiegna *et al.* 2015) or bacterial cell numbers (Schnyder *et al.* 2017) were also shown to increase with a higher bacterial strain or species diversity. In summary, the positive effect of biodiversity on ecosystem functioning is ubiquitous and well researched, whereas the mechanisms underlying this relationship are still hotly debated and the role of evolution is almost entirely unknown. As a consequence, the biodiversity–ecosystem functioning research continues to be a focal point of interest in environmental and biological sciences.

The importance of biodiversity–ecosystem functioning research is furthermore fueled by the current rates of biodiversity loss, which are higher than ever before (Barnosky *et al.* 2011) and threaten ecosystems all over the globe (Steffen *et al.* 2015). Anthropogenic pressure reduces more than only the cultural and emotional value (Bengtsson, Jones & Setälä 1997) of species richness *per se*, namely species loss results in a reduction of ecosystem functions that underpin valuable ecosystem

services, such as provisioning (e.g. timber production and fresh water) or regulating services, e.g. climate change mitigation (Cardinale *et al.* 2012).

BEF in grasslands

Research on the biodiversity–ecosystem functioning relationship (BEF) in grasslands started in the early 1990ies and since then a large body of studies accumulated showing the importance of grassland diversity on seasonal biomass production (productivity) and its stability over time or in response to extreme climatic events (among many others Tilman 1994; Hector *et al.* 1999; Allan *et al.* 2011; Cardinale *et al.* 2013; Gross *et al.* 2014; Isbell *et al.* 2015; Meyer *et al.* 2016). In these studies, two main mechanisms acting above and belowground have been proposed to play a crucial role in driving the positive BEF relationship. The first mechanism is named sampling effect, because diverse plant communities have a higher chance to include highly productive species than less diverse plant communities and therefore, are more likely to be more productive than less diverse plant communities (Loreau & Hector 2001). The increase in productivity at higher plant diversity has in addition been attributed to greater biomass providing more decomposable material at higher diversity (Fornara & Tilman 2008). Complementarity effects characterize the second mechanism. Complementarity effects can be separated into niche-based complementarity and positive species interactions, namely facilitation. Niche-based complementarity describes the situation when more diverse communities can be more productive and stable if the co-existing constituents of the community are complementary in their resource-use or in their specific pathogen susceptibility (Savage 1958; Silvertown 2004; Roscher *et al. 2008;* Mueller *et al.* 2013). Such complementarity, which leads to decreased competition and pathogen pressure in the community, can be mediated via diversification of the canopy structure and hence light and space use (Spehn *et al.* 2000; Allan *et al.* 2011), soil resource partitioning (Fornara & Tilman 2008; Roscher *et al.* 2008; von Felten *et al.* 2009), root depth

Fig. 1 | Biodiversity research in a grassland experimental field site. To quantify the biodiversity–productivity relationship, we harvested the aboveground biomass annually in spring and summer. The biomass was sorted according to species, which is a laborious procedure and requires knowledge in plant identification. Fieldwork is performed in sun and rain, with sunny weather being the more preferred climate. Data from this fieldwork are used in Chapters 1 and 2.

distribution (Mueller *et al.* 2013) and leaf mass distribution (Wacker *et al.* 2009). Facilitation is another mechanisms enabling more diverse communities to outperform such of lower diversity (Brooker *et al.* 2007). Here, it is because some species facilitate growth and survival of other species. For example, legumes are able to fix atmospheric nitrogen (Trydeman Knudsen *et al.* 2004) or to mobilize soil phosphorus (Li *et al.* 2007, 2014) and consequently increase the nutrient pool in the soil for other species.

Expanding the research focus to belowground interactions provided an additional explanation for the biodiversity–productivity relationship via negative plant–soil feedbacks (Mills & Bever 1998; van der Heijden, Bardgett & van Straalen 2008; Kulmatiski, Beard & Heavilin 2012a). Experimental manipulations of the soil organism composition have typically shown that accumulating species-specific pathogens decrease plant productivity at low diversity (Bever 1994; Schnitzer *et al.* 2011a). In contrast, at high diversity, pathogens promote species co-existence and productivity by density-dependent mortality (Petermann *et al.* 2008a). In contrast, positive plant–soil feedbacks are thought to promote dominance (van der Putten *et al.* 2013a). Such positive plant–soil feedbacks have often been attributed to arbuscular mycorrhizal fungi (AMF), ubiquitous soil-borne fungi able to form symbiotic relationships with plants. AMF have the potential to improve plant survival and growth under certain conditions by increasing nutrient uptake of the host plant (Jones & Smith 2004; van der Heijden *et al.* 2006), but the outcome of the interaction can vary from mutualism to parasitism (Kiers & Van Der Heijden 2006; Argüello *et al.* 2016).

Interestingly, Zuppinger-Dingley et al. (2014) showed that aboveground complementarity effects strengthen over time through selection for increased niche differentiation, relaxing interspecific competition in diverse plant communities but not in monocultures (Zuppinger-Dingley *et al.* 2014b). This finding revealed the important role of selection and evolution for BEF relationships, which is, however, not well resolved in its mechanistic detail. Furthermore, it is also not clear on which temporal scale the selective forces emerging in different communities of mixtures and monocultures are able to contribute to the evolution of the increased complementarity through time.

Natural selection and adaptation

Clearly, temporal dynamics are able to shape BEF relationships, as with time some community members go extinct, new species enter the system, interactions are lost and gained and species adapt to abiotic as well as biotic factors. For grassland systems, the positive effect of plant species richness on biomass production was found to increase over time (Cardinale *et al.* 2007; Fargione *et al.* 2007; Reich *et al.* 2012). During the last decade, evolutionary and ecological time scales converged, after Hairston introduced the term “rapid evolution” and defined it as “genetic change occurring rapidly enough to have a measurable impact on simultaneous ecological

change" (Hairston *et al.* 2005). However, grassland studies integrating a temporal aspect are sparse (Cardinale *et al.* 2007; Reich *et al.* 2012). Only recently, evolutionary mechanisms have come to the attention of researchers in plant systems (Zuppinger-Dingley *et al.* 2014b, 2015, 2016b; Kleynhans *et al.* 2016; Rottstock *et al.* 2017).

Within the same species, populations can adapt to a local environment, which is a well-described phenomenon called local adaptation (Linhart & Grant 1996; Leimu & Fischer 2008). A large number of studies found evidence in plants for local adaption to different abiotic environments (Schmid 1985; Joshi *et al.* 2001; Becker *et al.* 2006; Fox & Harder 2015), such as a dryer climate dominated by droughts (Franks, Sim & Weis 2007) or a change in soil conditions (Snaydon & Davies 1982; Gauthier, Lumaret & Bedecarrats 1998). The selective environment, however, can also be biotic, for example the presence of interspecific competition (Prati & Schmid 2000; Farrer & Goldberg 2011), bacterial (Parker 1995) or fungal (Johnson *et al.* 2010) mutualists or pathogens (Gilbert 2002). For example it was found that local populations of the grass *Andropogon gerardii* were adapted not only to the soil, but also to those mutualistic arbuscular mycorrhizal fungi which maximized the exchange of the most limiting resource (Johnson *et al.* 2010). There is also a body of research on the co-evolution between plants and pollinators. For example, rapid evolution was shown for a species of *Brassicaceae* in response to different pollinators (Gervasi & Schiestl 2017): within eleven generations of plant growth, populations of *Brassica rapa* evolved differently in response to different pollinators. In another study, plants evolved the ability to self-fertilize within five generations in response to pollinator loss (Bodbyl Roels & Kelly 2011). All these examples from plant research illustrate the strength of the selective pressures from biotic players in a plant community and emphasize the large potential of evolutionary processes shaping the biodiversity–productivity relationship in grasslands.

Plant–plant interactions, however, have received less attention and in particular one biotic selective environment has rarely been considered: community diversity. Here, the focus is not on the composition of the community, as in predator–prey interactions or pathogen defense mechanisms, but on the diversity of a given ecosystem. Diversity can act as selective environment and alter the fitness landscape, as was shown before (Kleynhans *et al.* 2016). In plants, few studies have considered species selection in a community environment. One of the first studies showing the evolution of mixture and monocultures types in grasslands was published in 2011 (Lipowsky *et al.* 2011). In 2014, Zuppinger-Dingley showed evolution of niche differentiation in plants selected for a mixed environment. In 2016, two studies focused each on a specific species, *Poa pratensis* (Kleynhans *et al.* 2016) and *Knautia arvensis* (Rottstock *et al.* 2017) to study the influence of a past community environment on a current environmental assay.

When plant populations are confronted with a novel environment, either because of range shifts or local climate changes, they have an array of options at hand to adapt (Ouborg, Vergeer & Mix 2006). A fast way to adapt is via a sorting-out of

suitable genotypes if there is sufficient standing genetic variation in a population (Fakheran *et al.* 2010). Furthermore, plants can adapt relatively quickly to a novel environment by phenotypic plasticity (Price, Qvarnstrom & Irwin 2003; Turcotte & Levine 2016), thus changing their morphology without changes in their DNA. Epigenetic mechanisms have also been suggested to enable adaptation (Bossdorf, Lipowsky & Prati 2008), especially in short-term evolutionary processes. Over longer time periods and across several generations, adaptation is also likely by genetic recombination and novel mutations (Anderson, Willis & Mitchell-Olds 2011).

Community evolution: definitions and mechanisms

Interactions between plants and animals have received a lot of attention and such two-way interactions for example between plants and pollinators, pathogens or herbivores have been studied extensively (Thorpe *et al.* 2011). The aim of this thesis was, however, to study interactions *among* plant species, which have been neglected in the past, due to the "diffuseness" of these interactions (Thorpe *et al.* 2011). To give these diffuse plant–plant interactions and their influence of eco-evolutionary processes a name, I use the term community evolution. Community evolution has been defined as the result of changes in gene frequencies among species of a community (Goodnight 1990a; Whitham *et al.* 2006). It assumes evolution to play out on the level of *entire communities*, i.e. on species performances *and their interactions,* leading to genetic changes in all or some of the species of the community. Currently there exist different definitions of what I refer to as community evolution and there is confusion around the terms community evolution, community selection, group selection, diffuse co-evolution and community genetics. In the following paragraph, I aim to shed light on the many definitions and to make my use of the term clear for the rest of this thesis.

Fig. 2 | Common garden experiment in the glasshouse. Plant communities were grown in a number of 2-species combinations and in monocultures from February to September 2015 in pots in a glasshouse at the University of Zurich. In this experiment (see Chapter 3), I addressed the question whether 12 years in a mixture or monoculture selective environment can lead to the differentiation of monoculture and mixture types as visible in their phenotype.

The term community evolution has a long history in literature to explain community-level evolutionary changes. Depending on the research area, however, it was used differently. For example, in the book *Community evolution and the origin of mammals*, the palaeontologist Olson (1966) defined community evolution as "crudely analogous to organic evolution". He used the term community evolution to emphasize that over a long period of time (millions of years), communities can be stable, split, merge (analogue to hybridization), or converge. Community evolution was then later defined as "natural selection leading to phenotypic change at the community level" (Wilson 1997; Whitham *et al.* 2003), before, in 2006, Whitham defined community evolution as "a genetically based change in the ecological interactions that occur between species over time" (Whitham *et al.* 2006).

Evolution per definition has a genetic base; hence it seems obvious that community evolution is accompanied by the field of community genetics. The concept of community genetics was introduced by Antonovics (1992) because he saw a need for a new level of analysis that goes beyond the population level. He viewed community genetics as an approach to look at evolutionary genetic processes that occur among interacting populations of species within communities. He claimed that community genetics would "free us […] from the reciprocity that co-evolutionists would choose for their own discipline" (Antonovics 1992). In this sense, the idea of community genetics underlies community evolution on a mechanistic level and is the study of the genetics of species interactions and their ecological and evolutionary consequences. In 2003, Neuhauser *et al*. described community genetics as "a synthesis of community ecology and evolutionary genetics; it directly assesses the interplay between genetic variation and community dynamics to develop a mechanistic understanding of the evolution of organisms in the context of the communities that they occupy" (Neuhauser *et al.* 2003). An important point explained by Neuhauser *et al.* (2003) is the fact that in community genetics, the premise is that the genetic composition of populations within species may vary substantially between communities. The conceptual framework presented in that paper is a good base for our understanding of both community genetics and community evolution.

In his seminal papers *Experimental Studies of Community Evolution I & II* from 1990, Goodnight used community selection as a prerequisite for community evolution and defined it as "differential proliferation and/or extinction of communities […], which can result in genetic changes in all of the species within the community by acting on the interaction among species" (Goodnight 1990b; a). He argued that community selection acts when populations of more than one species are involved in coevolving interactions comprising a community, which itself is under selection. However, it is important to note that other authors have defined community selection as an *assembly process*, referring to the selection of parts of a bigger community because of selective pressures (Kendeigh 1945; Mendes *et al.* 2014), which is an entirely different use of the term community selection. Here, community selection describes the local segregation of species into divergent communities, for example due to environmental conditions (Kendeigh 1945). More as an anecdotal side note, the definition of community selection has even stretched as far as the choice of which

vegetation communities cattle prefer to graze on (Gordon 1989).

To add to the confusion, the term "group selection" has been used widely to describe natural selection acting on a group of individuals (Wade 1977; Wilson 1983) as opposed to natural selection acting on an individual level. Group selection was defined as "that process of genetic change brought about or maintained by the differential extinction and/or proliferation of populations" (Wynne-Edwards 1962; Wade 1977). Hence, group selection results in population-level evolutionary consequences, while our use of the definition of community evolution has community-level consequences. In this regard, community evolution (and community selection sensu Goodnight 1990) is an extension of the concept of group selection; in the same way as community ecology is an extension of population ecology.

Diffuse co-evolution was termed by Janzen (1980) describing the situation "when an array of populations […] generate a selective pressure as a group." He viewed diffuse co-evolution as an extension to simple co-evolution, in which case only two populations interact and exert selective pressures on each other (Janzen 1980). A few years later, Fox (1988) stated that despite its broad acceptance, there was little empirical evidence for diffuse co-evolution.

Most knowledge on community evolution stems from experiments with small organisms (but see Goodnight 1990a), which are easier to manipulate and have shorter generation times, such as bacteria or plankton (Yoshida *et al.* 2003; Lawrence *et al.* 2012; Fiegna *et al.* 2014, 2015). It is conceivable that these evolutionary principles can be extended to communities of other species, such as plants. In this thesis, I used perennial plant species, which have a long generation time and undergo few generations over the course of a typical multi-year experiment. This does not make evolutionary processes impossible though. By a sorting-out mechanism of the standing genetic variation, well-suited genotypes for a specific community can be selected within only a few generations (Barrett & Schluter 2008; Fakheran *et al.* 2010).

Evolutionary mechanisms: genetic and epigenetic processes

At the basis of evolution are changes in the DNA sequence of an individual. Variation in the DNA sequence between individuals of the same species can happen by spontaneous mutations, sexual reproduction or gene flow. Natural selection can act on these changes, and depending on the change, selection will favor this type of variation. Genetic variation is hence needed for evolution and the basis for speciation, adaptation and extinction. However, there is a second mechanism with the power to exert changes on the DNA, but without changing the DNA sequence: the methylation of DNA. This methylation belongs to a larger group of epigenetic processes (Greek: *επί-* over, outside of, around), which are hereditary forms of DNA change happening more rapidly than base pair changes and substitutions (Verhoeven, vonHoldt & Sork 2016). Epigenetic processes have been claimed to be relatively common in plants (Bossdorf *et al.* 2008) and several studies have studied the effect of methylation on plant phenotypes in glasshouse experiments by applying a de-

methylation agent (Vergeer, Wagemaker & Ouborg 2012; Wilschut *et al.* 2016). However, the importance of epigenetics in natural populations is still unclear. Recently, a new method was developed, enabling one to gain information about methylation and single nucleotide polymorphisms (SNPs) in the same sequencing run and for the same sample (van Gurp *et al.* 2016). This "representative reduced bisulfite sequencing" (RRBS) technique enables the study of epigenetic and genetic variation on the same sequence of gene material. In this way, it is possible to elucidate whether epigenetic variation is due to underlying genetic variation or independent of it. Furthermore, by applying this method it is possible to know whether only epigenetic, or also genetic changes, are involved in driving phenotypic changes. Here, I used this novel method to test whether community diversity could act as a selective force leading to the evolution of populations within the same species exhibiting distinct diversity–productivity relationships. In particular, I wanted to find out whether genetic or epigenetic factors were driving the differentiation of plant communities into mixture types (exhibiting positive biodiversity effects) or monoculture types (exhibiting negative biodiversity effects) within the same species.

The Jena Experiment

The Jena Experiment field site is located in the floodplain of the river Saale (see Fig. 3) in Jena, Thuringia, Germany, 51˚N, 11˚E, 135 m a.s.l. Mean annual temperature of the area is 9.9 ˚C and mean annual precipitation is 610 mm (Hoffmann & Bivour 2014). The Jena Experiment is a long-term biodiversity field experiment where 60 Central European grassland species are grown in a number of species combinations since 2002 (Roscher *et al.* 2004a). It is the longest-running such experiment in Europe and globally only rivaled by a few other large biodiversity experiments (e.g. the BioCON of the University of Minnesota in Cedar Creek, USA or the joint Chinese-German-Swiss research project BEF-China in China). The Jena Experiment represents a unique opportunity to perform experiments incorporating an evolutionary aspect. Therefore, in 2010 our research group installed experimental plots to study the influence of community evolution on ecosystem functioning, i.e. plant productivity. Plant communities with a history of co-selection in mixtures or monocultures in Jena since 2002 were planted adjacent to plant communities consisting of plants without such community history (see Methods of Chapters 1 and 2) and the diversity–productivity relationship was studied over four years.

Fig. 3 | Aerial photograph of the Jena Experiment. Plant communities are grown in a number of species compositions and diversity levels in experimental plots since 2002. For this thesis, within the main experiment (visible as squares of different shades of green and brown) a smaller experimental subunit of 2 x 2 m was installed in 2010. Figure from www.the-jena-experiment.de, accessed on 4 January 2016.

Research questions and thesis outline

This thesis addresses questions in community ecology, integrating an evolutionary perspective. In the first two Chapters, I asked whether there is evidence for community evolution in experimental grassland communities and how this community evolution influences both ecosystem functioning and stability. Hence the two leading research questions for the first half of the thesis are:

i) How does community evolution alter productivity of grassland communities?

ii) How does community evolution alter the stability of ecosystems?

Chapters 3 and 4 focus on a species-level experiment conducted in the glasshouse. I assessed whether community diversity can act as a selective environment and lead to the differentiation of monoculture and mixture types between population of the same species. The main research questions for the second part of the thesis were:

iii) Is there evidence for rapid evolution into mixture and monoculture types within a species in a grassland experiment?

iv) Are genetic (DNA sequence) or epigenetic factors (methylation) driving the differentiation into monoculture and mixture types within a species?

In the last Chapter Terhi Hahl and I address the importance of plant–soil feedbacks using a pot experiment with single individuals in the glasshouse. The aim was to test whether plants from either monoculture or mixture selection history would differ in their ability to form symbiotic relationships with arbuscular mycorrhizal fungi. In addition, we investigated whether the origin of the AMF community (either mixture or monoculture experimental plots) differentially influenced the aboveground biomass of plant individuals of either mixture or monoculture history.

References of this Introduction and the Discussion are merged and listed in the bibliography at the end of this thesis.

CHAPTER ONE

Community evolution increases plant productivity at low diversity

Community evolution increases plant productivity at low diversity

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Currently in revision for Ecology Letters

Author contributions

B.S., D.B.F. and G.B.D.D. conceptualized the project; S.J.V.M., T.H., and D.Z.D. carried out the experiment; B.S., C.W., S.J.V.M. and T.H. analysed the data; D.B.F. analysed the TRFLP data; B.S., S.J.V.M., T.H. and C.W. wrote the first draft of the manuscript. All authors contributed to the final manuscript.

ABSTRACT

Species extinctions from local communities can negatively affect ecosystem functioning. Ecological mechanisms underlying these impacts are well studied but the role of evolutionary processes is rarely assessed. Using a long-term field experiment, we tested whether natural selection in plant communities increased the effects of biodiversity on productivity. We re-assembled communities with 8-year co-selection history adjacent to naïve communities with identical species composition but no history of co-selection. Mixtures of two to four co-selected species were more productive than their corresponding naïve communities over four years in soils with or without co-selected microbial communities. At the highest diversity level of eight plant species, no such differences were observed. Our findings suggest that plant community evolution can lead to rapid increases in ecosystem functioning at low diversity but may take longer at high diversity. This effect was not modified by treatments that simulated additional co-evolutionary processes between plants and soil organisms.

Key words: biodiversity, community evolution, co-selection, ecosystem functioning, grassland species, Jena Experiment, plant productivity, soil organisms

INTRODUCTION

A large number of experiments have shown that species richness positively influences ecosystem functioning, in particular plant biomass production (Tilman, Lehman & Thomson 1997; Balvanera *et al.* 2006; Cardinale *et al.* 2007; Reich *et al.* 2012; Cardinale *et al.* 2012; Meyer *et al.* 2016). These biodiversity effects have been explained by sampling effects that increase the chance of including productive species in diverse communities (Tilman *et al.* 1997; Huston 1997) or by complementary effects between species, which allow mixtures to extract resources from the environment more efficiently (Roscher *et al.* 2008; Mueller *et al.* 2013). Furthermore, diversity-dependent reductions in soil fertility (Fornara & Tilman 2008) or density-dependent accumulations of specialist pathogens over time (Schnitzer *et al.* 2011a) have been shown to contribute to decreasing productivity at low plant diversity and in plant monocultures.

Complementarity effects between co-occurring species increase over time (Cardinale *et al.* 2007; Fargione *et al.* 2007; Reich *et al.* 2012; Meyer *et al.* 2016). Evidence that this might be due to evolutionary processes in plant communities has been found in a glasshouse experiment comparing the performance of populations selected in monocultures vs. diverse plant communities in newly assembled test monocultures and two-species mixtures (Zuppinger-Dingley *et al.* 2014b). This suggests that community evolution may shape diversity–productivity relationship more generally, which could be tested if entire communities of co-selected plant species would be compared with communities of the same plant species but without co-selection history. Community evolution has been defined as genetically based changes among species constituting the community, which alter species performances and interactions (Whitham *et al.* 2006). Such changes may occur via genetic recombination, mutations (Anderson *et al.* 2011), or a sorting-out from standing genetic variation through differential survival and growth of individuals (Fakheran *et al.* 2010). Natural selection can lead not only to changes in gene frequencies in populations within species, but evolution at the level of communities can in addition lead to correlated changes in gene frequencies in multiple species (Whitham *et al.* 2006) in response to one another or to co-varying environmental conditions. But empirical evidence for community evolution so far has only been demonstrated in bacterial communities (Lawrence *et al.* 2012; Fiegna *et al.* 2014, 2015) and not yet in higher plants. Here we report results from a field experiment where we tested whether plant community evolution influences plant community productivity.

Recent evidence suggests selection of particular genotypes from the total genetic pool of a species may affect ecosystem functioning in field experiments (Strauss *et al.* 2008; Lipowsky *et al.* 2011; Lau & Lennon 2012; Kleynhans *et al.* 2016; Rottstock *et al.* 2017). We propose that selection of genotypes from the gene pool of entire communities may affect ecosystem functioning if non-random niche or trait changes in response to other phenotypes in the community result in reduced niche overlap and a more complete use of biotope space (Dimitrakopoulos & Schmid 2004; Jousset *et al.* 2011), thus leading to increased plant community productivity.

We therefore compared the productivity of plant communities assembled from plants which have co-occurred for eight years in a long-term grassland biodiversity experiment (the Jena Experiment, see Roscher *et al.* 2004) with the productivity of plant communities of identical species composition, but without any co-occurrence history ("naïve communities"). The naïve plants were obtained from the seed supplier of the original seeds used to establish the Jena Experiment. We used experimental plant monocultures and 2-, 4- or 8-species mixtures with twelve different species compositions for each diversity level.

Plant community evolution in the field may also depend on the local environment, such as the soils in which co-evolution with soil microorganisms occurred. For instance, plant–soil feedback experiments have shown that soil biota change in response to different plant species, which can in turn modify the composition and productivity of plant communities (Klironomos 2002a; Kardol *et al.* 2007; Wagg *et al.* 2015). To assess whether additional co-evolutionary processes between plants and soil organisms modified plant community evolution, we grew the selected and naïve plant communities in soils with co-selected soil organisms (native soil) and with external soil organisms (neutral soil; see Methods and Fig. S1). Community-level plant productivity was measured each year from 2012 to 2015 by collecting species-specific aboveground biomass at the time of peak biomass in spring (see Methods).

METHODS

Study site

The present study was conducted at the Jena Experiment field site (Jena, Thuringia, Germany, 51˚N, 11˚E, 135m a.s.l.) from 2011 to 2015. The Jena Experiment is a long-term biodiversity field experiment located in the floodplain of the river Saale where 60 Central European grassland species have been grown in a number of species combinations since 2002 (Roscher et al. 2004).

Community-evolution treatment (plant history)

The 48 experimental plant communities of this study included twelve monocultures (of which one had to be removed from all analyses because it was planted with the wrong species), twelve 2-species mixtures, twelve 4-species mixtures and twelve 8-species mixtures. We used two community-evolution treatments; plants with eight years of co-selection history in 48 different plant communities in the Jena Experiment (communities of co-selected plants) and plants without such co-selection history in the Jena Experiment (naïve communities). The plant seeds of naïve communities were obtained from the same commercial seed supplier (Rieger Hofmann GmbH, in Blaufelden-Raboldshausen, Germany) as the seeds used for the establishment of the original communities of the Jena Experiment. This supplier collected plants of the different species at field sites in Germany and propagated them

for at least five years in monoculture, reseeding them every year. Seeds of communities of co-selected plants were produced in an experimental garden in Zurich, Switzerland, from cuttings that had been made in the Jena Experiment and were then planted in Zurich in the original species combination in plots fenced with plastic netting to reduce pollination between communities. To obtain sufficient numbers of seeds from communities of co-selected plants, a small number was additionally collected directly in the plots of the Jena Experiment. All these seeds were thus offspring of plant populations that had been sown in 2002 and grown until 2010 in plots of the Jena Experiment.

The seeds of communities of co-selected plants and naïve communities were germinated in potting soil (BF4, De Baat; Holland) in mid-January 2011 in a glasshouse in Zurich. In March 2011, the seedlings were transported back to the field site of the Jena Experiment and planted within 2 x 2 m subplots of the original plots (Fig. S1). There were four 1 x 1 m quadrats with different soil treatments in each (see next section). Each quadrat was further divided into two 1 x 0.5 m halves. The seedlings of communities of co-selected plants were transplanted into one half and seedlings of naïve communities into the other half of each quadrat at a density of 210 plants per m^2 with a 6-cm distance between individuals in a hexagonal pattern (Fig. S1). Species were planted in equal proportions, but if a species was no longer present in an original plot of the Jena Experiment it was excluded from both communities of co-selected plants and naïve communities. Five plant species were excluded in total. The seedlings received water every second day for six weeks after transplanting to ensure the plants established.

Soil treatment

Within each 2 x 2 m subplot of the 48 plots of the Jena Experiment used for the present study, the original plant cover was removed in September 2010 (and used for the plant propagation in the experimental garden in Zurich, see previous section), and the soil was excavated to a depth of 0.35 m and sieved. To minimize exchange of soil components between quadrats within subplots and with the surrounding soil, two 5-cm layers of sand were added to the bottom of the plots and separated with a 0.5 mm mesh net. The borders of the quadrats and the subplots were separated by plastic frames (Fig. S1). Using the excavated original soil from each of the plots, four soil treatments were prepared. First, half of the soil (approximately 600 kg per plot) was gamma-sterilized to remove the original soil community. Half of the gamma-sterilized soil was then inoculated with 4 % (by weight) of live sugar-beet soil and 4 % of sterilized original soil of the corresponding plot ("neutral soil" obtained by inoculation). Live sugar-beet soil was added to create a natural, but neutral soil community and was previously collected in an agricultural sugar-beet field not associated with the Jena Experiment, but with comparable soil properties. The other half of the gamma-sterilized soil was inoculated with 4 % (by weight) of live sugar-beet soil and 4 % of live original soil of the corresponding plot ("native soil" obtained by inoculation). The other half of the soil was unsterilized and used for the other two

soil treatments. Half of this soil was filled back into one quadrat of the corresponding plot ("native soil"). The other half of the unsterilized soil was mixed among all plots and filled into the remaining quadrats. This fourth soil treatment was abandoned after two years because the plant community was excavated for another experiment. Therefore, this treatment is not included in the present study.

Before the soils were added into the quadrats in December 2010, they were rested in the field in closed bags to allow for the soil chemistry to equalize and to encourage soil biota of the inocula to colonize the sterilized soil before planting. After the soil was added, all quadrats were covered with a net and a water permeable black sheet to avoid spilling between quadrats until the seedlings were transplanted in March 2011.

Data collection

We maintained the test communities by weeding three times a year and by cutting the plants twice a year at typical grassland harvest times (late May and August) in central Europe. To measure productivity, we harvested plant material 3 cm aboveground from a 50 x 20 cm area in the centre of each half-quadrat, sorted it into species, dried it at 70°C and weighed the dry biomass.

SLA measurements

At the end of the experiment, in May 2015, we measured specific leaf area (SLA) for 30 species in neutral soil. For each species, we collected up to 20 representative leaves (depending on the leaf size of the species) from four individuals and measured the leaf area by scanning fresh leaves with a Li-3100 Area Meter (Li-cor Inc., Lincoln, Nebraska, USA) immediately after harvest and determining the mass of the same leaves after drying.

T-RFLP assay

Terminal restricted fragment length polymorphism (T-RFLP) targeting the 16S RNA was used to characterize the composition of the soil bacterial communities (Liu *et al.* 1997). In April 2011, four soil samples per quadrat were extracted and pooled to assess the establishment of soil microbial communities and to test whether soil treatments were distinct. In 2012, a further set of soil samples was taken and analysed to confirm the establishment of different soil biotic treatments. T-RFLP soil analyses revealed that bacterial communities of the soil treatments remained distinct: each soil treatment had characteristic bacterial compositions both one and two years after planting, with some overlap (Table S3).

Statistical analysis

We analysed the data from the four spring harvests 2012, 2013, 2014 and 2015, which corresponded to peak aboveground plant biomass values. We analysed plant biomass (g/m^2) as a function of the design variables using mixed models and

summarized results in analyses of variance (ANOVA) tables (e.g. Table S1). Significance tests were based on approximate F-tests using appropriate error terms and denominator degrees of freedom.

The fixed terms in the model were species richness of the original plots of the Jena Experiment (factor with 4 levels: facSR), year of harvest (factor with 4 levels: Har), soil treatment (factor with 3 levels: SH), community-evolution treatment (communities of co-selected plants vs. naïve communities: PH) and interactions of these. The random terms were plot, quadrat, half-quadrat and their interactions with year of harvest. Statistical analyses were conducted using the software R, version 3.2.3 (R Core Team 2015). Mixed models using residual maximum likelihood (REML) were fitted using the package ASReml for R (Butler 2009).

Within-species variation in SLA was calculated as the within-species variance component for each community (residual mean square after fitting species). We had insufficient trait data to test for increased between-species variation in communities of co-selected plants containing a mixture of species.

The calculation of operational taxonomic units (OTUs) from the T-RFLP raw data (restriction enzyme products) was done using the T-RFLP processing software T-REX (Culman *et al.* 2009) for each soil treatment and year separately and the soil-specific outputs were then compared with an analysis of similarities (anosim() function of the vegan package (Oksanen *et al.* 2016).

RESULTS

Overall, for each doubling of species richness community aboveground biomass increased by 100 $g \cdot m^{-2} \cdot y^{-1}$, a typical value for grassland biodiversity experiments (Hector *et al.* 1999). In general, communities of co-selected plants were more productive than naïve communities of the same species composition. The significant interaction between species richness and community-evolution treatment or in short plant history ($F_{3,191.2} = 2.77$, $P = 0.043$; Table S1a) indicated that this was mainly due to increased productivity of 2- and 4-species mixtures and a smaller increase in monocultures of co-selected plants. In contrast, 8-species mixtures of co-selected or naïve plants were equally productive (Fig. 1a). The calculated relative productivity (percentage of the mean productivity of 8-species mixtures for each plant history-by-soil treatment-by-year combination) confirmed that especially 2- and 4-species mixtures of co-selected plants increased productivity relative to 8-species mixtures ($F_{3,191.9} = 2.90$, $P = 0.036$; Fig. 1b; Table S1b). The positive effect of community evolution on relative productivity was significantly larger in 2- and 4-species mixtures than in monocultures ($F_{1,43.7} = 6.37$, $P = 0.015$ for the interaction between plant history and the contrast of "2- or 4- species mixtures vs. others"). The differences in relative productivity between communities of co-selected plants and naïve communities increased over time for these low-diversity mixtures as well as for monocultures in all three soils (Fig. 2). For monocultures, this was due to the deteriorating performance of naïve plants, possibly due to the accumulation of soil

pathogens, whereas for 2- and 4-species mixtures it was due to an increasing relative performance of communities of co-selected plants.

To test whether the communities of co-selected plants were particularly productive in 2- and 4-species mixtures at the beginning of the Jena Experiment (i.e. when they were "naïve" communities themselves), we compared the productivity data of 2003–2006 with the data of 2012–2015. To standardize for differences in overall productivity between time periods we again used relative productivity (percentage of mean of 8-species mixtures per year). The plant communities were established in neutral soil in 2002 at the beginning of the experiment. We therefore used only data from neutral soil from 2012 to 2015. The communities of co-selected plants were significantly different in their response compared to the two types of naïve communities because of their increased relative productivity in 2- and 4-species mixtures ($F_{1,46.5} = 5.73$, $P = 0.021$ for the interaction of plant history with the contrast "2- or 4-species mixtures vs. others"; Fig. S2). Differences between the communities of the naïve ancestors of the co-selected plants and our current re-assembled naïve plant communities were small and not significant ($F_{1,46.1} = 0.23$, $P = 0.637$ for the interaction of the contrast "naïve ancestors vs. current naïve communities" with the contrast "2- or 4- species mixtures vs. others").

Plant community productivity was initially greater in inoculated soils, in particular at high diversity, which was reflected in an overall main effect of soil treatment and significant interactions with year, and with year and species richness (Table S1). This was probably caused by the nutrient flush associated with gamma-sterilization of the soil (Gebremikael *et al.* 2015). But we found no evidence that our soil treatments modified the differences in biodiversity effects between communities of co-selected plants and naïve communities ($F_{1,183} = 0.27$, $P = 0.847$ and $F_{1,183.8} = 1.401$ $P = 0.244$ for the three-way interactions of plant history with species richness and the soil-treatment contrasts neutral vs. native and sterilized native vs. unsterilized native, respectively).

To explore potential mechanisms for the increased biodiversity effects in 2- and 4-species mixtures of co-selected plants, we calculated the proportional increase (decrease) in plant productivity for each community composition and soil treatment as the log ratio between communities of co-selected plants and naïve communities (Fig. 3). As expected, there was no increase in productivity in 8-species mixtures, but a strong increase in 2-species mixtures followed by 4-species mixtures (which had a higher absolute increase than 2-species mixtures, see Fig. 1a) and monocultures. Using contrasts between the different diversity levels, we could confirm that the three low diversity levels were significantly different form the 8-species mixtures ($F_{1,37.1} = 5.34$ and $P = 0.026$). Among the three low diversity levels, the 2-species mixtures had significantly greater log ratios than 4-species mixtures and monocultures ($F_{1,39.2} = 4.44$, $P = 0.042$).

Next, we tested whether the presence of particular plant functional groups influenced the increase in productivity in communities of co-selected plants at the 2- and 4-species richness levels; especially as legumes are known to drive over-yielding in grasslands (Spehn *et al.* 2002). The presence of legumes and other plant functional

groups, however, did not provide any further explanation for our results. Species-level productivity within communities was higher for the majority of plant species with a co-selection history, irrespective of functional-group identity (Fig. 4). Naïve communities showed more even species abundance distributions ($F_{1,132.2} = 4.28$, $P = 0.041$; Table S2), mainly due to the lower evenness of communities of co-selected plants in the unsterilized native soil treatment (Fig. S3). Over the course of the experiment, evenness decreased similarly in communities of co-selected plants and naïve communities (Table S2).

Finally, we analysed changes in within-species trait variation along the species richness gradient as a potential mechanism contributing to the difference in productivity between communities of co-selected plants and naïve communities (Siefert *et al.* 2015). Within-species variation in specific leaf area (SLA) decreased for communities of co-selected plants and increased for naïve communities with increasing species richness (Fig. 5; $F_{1,69.2} = 4.87$, $P = 0.031$ for interaction of log species richness with plant history).

DISCUSSION

Our results show that eight years of community evolution in a biodiversity experiment can increase biodiversity effects on community productivity, suggesting that this may at least in part explain why biodiversity effects commonly increase over time in such experiments (Cardinale *et al.* 2007; Fargione *et al.* 2007; Reich *et al.* 2012; Meyer *et al.* 2016). The greater productivity in communities consisting of co-selected plants compared to communities consisting of naïve plants was particularly evident in communities comprised of two or four species. One might claim that these effects were because we purchased the plant material of co-selected and naïve plants at two different points in time. We argue that this is not the case for the following reasons. First, co-selected and naïve plants were obtained for 52 different species and for each of them there were different community-specific co-selection histories. Second, 8-species mixtures with and without co-selection history showed the same productivity. In other words, because the positive effect of the community-evolution treatment was not statistically evident in the 8-species mixtures but strong in 2- and 4-species mixtures, this effect was unlikely simply due to initial differences in plant material.

Why was the community-evolution treatment not effective at the highest richness level tested? It is conceivable that selection pressure was dampened in communities where more than four species co-occurred. For instance, during initial establishment in a diverse community, each individual can have a entirely different set of immediate neighbours that could constrain the consistency in the selection pressure on individuals within a community. With fewer species in a mixture, the potential for the evolution of increased complementarity between plant species should be greater, given the relative constancy of the neighbours any given plant experiences. The greater proportional (but not absolute) increase of productivity in communities of

co-selected plant species at the 2- than at the 4-species richness level, and the absence of such an increase at the 8-species richness level, are compatible with the idea that evolution for co-adaptation is stronger at low than at high diversity. At low diversity, intraspecific densities are higher and thus the chance for a uniform selection pressure across all intraspecific individuals is greater. As a consequence, there might be an upper limit of species richness beyond which selection is unlikely to strengthen biodiversity effects (Cardinale *et al.* 2012). Additionally, community evolution leading to increased plant growth and productivity in diverse mixtures may be at the expense of reduced pathogen defence (Lemmermeyer *et al.* 2015).

The performance of the naïve communities in the current study over the four years was comparable to the initial performance of the ancestral community of the co-selected plants (2003–2006). This similarity supports the view that the observed results at 2- and 4-species richness levels in communities of co-selected compared with communities of naïve plants are likely due to diversity-dependent community evolution. Indeed, the naïve communities did not catch up with the communities of co-selected plants during the course of the current experiment and differences in productivity from 2012 to 2015 even increased between the two community-evolution treatments (Fig. 2). With regard to underlying evolutionary mechanisms, this suggests that in our study community evolution was not or at least not solely due to an immediate sorting out of genotypes from standing variation (Fakheran *et al.* 2010) during seedling establishment and initial growth.

The driving force behind community evolution for greater productivity at low diversity could have been related to particular species compositions (Zuppinger-Dingley *et al.* 2014b). There was, however, no evidence for any plant functional-group specific effect typically found in other contexts of biodiversity–ecosystem functioning research (Hooper & Vitousek 1997; Spehn *et al.* 2002). In fact, the majority of species produced greater biomass in communities of co-selected plants and evenness was only slightly reduced in these communities compared with communities of naïve plants.

Intraspecific variation in SLA decreased in communities of co-selected plants and increased in naïve communities with increasing species richness (Fig. 5), a result in line with previous findings for SLA in grassland species (Gubsch *et al.* 2011). The increased within-species variation in monocultures suggests an evolutionary broadening of niches to benefit from a wider range of light conditions. In contrast, within-species trait variation may be less important in mixtures, due to the inherently lower intraspecific density at greater richness. The narrowing of within-species variation with increasing diversity in communities of co-selected plants could be an expected consequence of character displacement between species (Zuppinger-Dingley *et al.* 2014b). In relative terms, it seemed that species in naïve communities had not yet responded to different diversity treatments with an adjustment of within-species variation in the four years of this study. A more heterogeneous biotic environment may have caused their higher variation at high diversity.

Selected plants also had greater productivity than naïve plants in monoculture. The adaptation of selected plants to monoculture environments could have been due to the evolution of increased (belowground) pathogen defence (Zuppinger-Dingley *et al.* 2016b) or greater niche width (Bazzaz 1996). Assuming soil-borne plant pathogens accumulated over time (Schnitzer *et al.* 2011a), in particular in the initially sterilized treatments, the decrease in monoculture productivity in naïve communities (Fig. 2) would be consistent with the hypothesis of increased pathogen defence in selected communities (Zuppinger-Dingley *et al.* 2016b). Assuming a correlation between resource-uptake and trait-based niches (Roscher *et al.* 2015), the increase in within-species variation in SLA in monocultures of selected plants (Fig. 5) would be consistent with the second explanation related to niche width.

Positive plant diversity–productivity relationships may not only be driven by complementary resource use, and thus increased performance at high diversity (Roscher *et al.* 2008; Mueller *et al.* 2013), but also by pathogen accumulation in the soil and thus reduced performance at low diversity (Schnitzer *et al.* 2011a). Previous studies in the context of biodiversity–ecosystem functioning research have reported negative plant–soil feedbacks in native as opposed to neutral soils (Klironomos 2002a; Petermann *et al.* 2008a; Cortois *et al.* 2016). Consequently, an increase of biodiversity effects during community evolution could also be due to the presence of co-selected soil biota. In our study, however, the outcome of the community-evolution treatment in mixtures was largely independent of the presence of co-selected soil biota. The generally lower productivity for both communities of co-selected plants and naïve communities in native soil, and with time in neutral soil, may have occurred through nutrient depletion or pathogen accumulation in all soil treatments. It is conceivable that co-evolution of plants with soil biota in our experimental systems was not effective because the large population sizes and short generation times of most soil organisms contributed to the re-assembly and fast evolution of soil communities (Lau & Lennon 2012). Another explanation could be that microbes were dispersed via wind-blown particles to adjacent plots thereby potentially making the microbial communities less different in composition than if the plots would have been separated more in space.

Changes in the performance of individual species selected in different species diversity levels and tested under experimental abiotic or biotic conditions have been observed in previous studies (Lipowsky *et al.* 2011; Zuppinger-Dingley *et al.* 2014b; Kleynhans *et al.* 2016; Rottstock *et al.* 2017). In our study, we demonstrated for the first time that changes in the performance of entire plant communities over time depend on a history of co-selection among the plants species of the assembled mixtures. We suggest that these changes are the result of community evolution because they were maintained through seed production in an experimental garden and propagation of seedlings in a glasshouse to the replanting of communities in the field. However, we cannot exclude maternal carry-over and epigenetic changes (Verhoeven *et al.* 2016) as additional potential evolutionary mechanisms. Independent of the mechanism, an ecosystem with individuals adapted to optimize the use of the local resources by reducing interspecific competition will be a well-functioning and

sustainable system. Our new findings suggest that it is not sufficient to preserve species outside a community context for the conservation of biodiversity and its beneficial influence on ecosystem functioning and services. To protect species interactions and ecosystem functioning more efficiently, novel strategies should consider the conservation of entire communities or at least subsets of these. Our results emphasize that this is especially critical for less diverse communities, which may already suffer from the loss of some of their constituents.

ACKNOWLEDGEMENTS

Thanks to the Jena Experiment for providing infrastructure and help, to D. Trujillo and M. Furler for technical assistance and to H. Martens for the lab work for the T-RFLP analyses. Thanks to V. Yadav for the establishment of the plots. We thank Tim Paine, Marc Cadotte and Mark van Kleunen for helpful comments on an earlier draft. This study was supported by the Swiss National Science Foundation (grant numbers 130720, 147092 and 166457 to BS) and the University Research Priority Program Global Change and Biodiversity of the University of Zurich. The Jena Experiment is funded by The Deutsche Forschungsgemeinschaft (DFG, German Research Foundation, FOR1451).

AUTHORSHIP

BS, DFBF and GBDD conceived the project; DZ-D set up the experiment; SJVM, TH and DZ-D carried out the experiment; BS, CW, SJVM and TH analysed the data; DFBF analysed the TRFLP data; BS, SJVM, TH and CW wrote the first draft of the manuscript. All authors contributed to the final manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

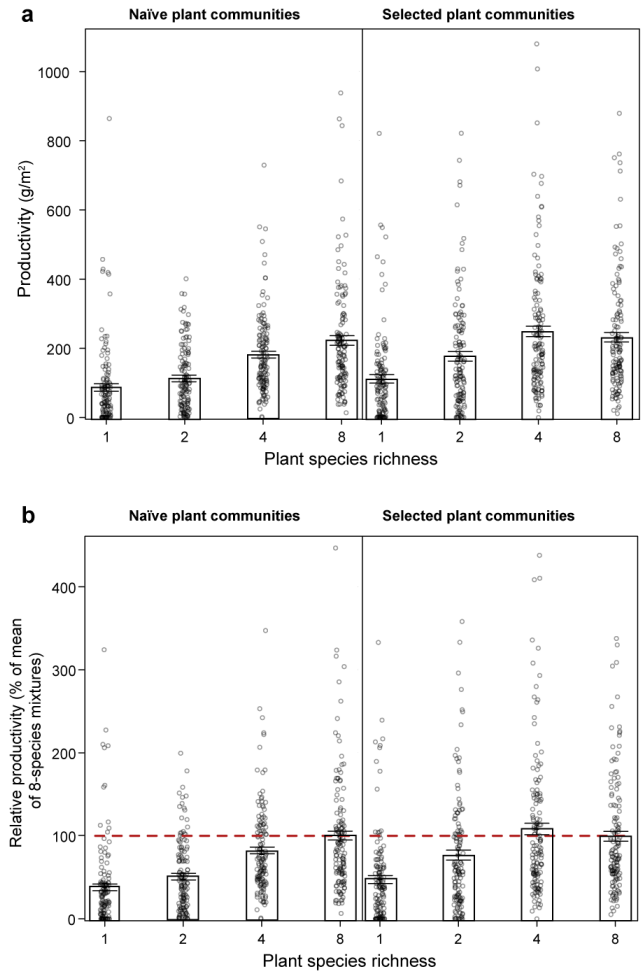

Figure 1 Community productivity for naïve communities and communities of co-selected plants at different species-richness levels. **(a)** Peak community aboveground biomass (g/m^2). Communities of co-selected plants (right panel) had slightly increased productivity in monocultures, more strongly increased productivity in 2- and 4-species mixtures, but similar productivity in 8-species mixtures as naïve communities (left panel). **(b)** as in (a) but showing relative productivity (% of mean productivity of 8-species mixtures per plant history-by-soil treatment-by-year combination). Means and standard errors are shown. Raw data plotted as points.

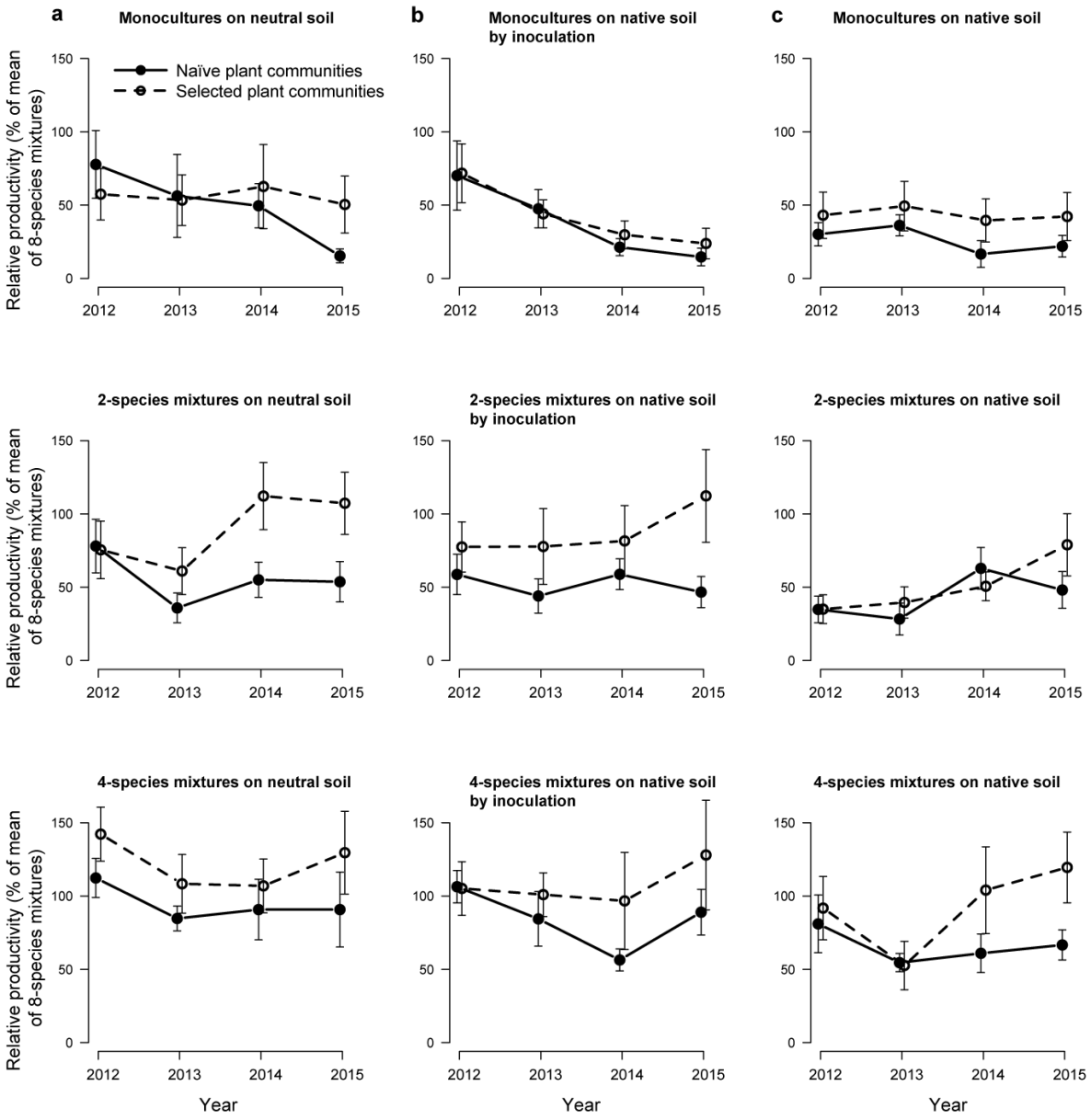

Figure 2 Relative productivity (% of mean of 8-species mixture) of communities of co-selected plants (dashed lines, open circles) and naïve communities (solid lines, closed circles) in monocultures and 2- and 4-species mixtures in **(a)** neutral soil (sterilized soil with neutral inoculum) **(b)** native soil obtained by inoculation (sterilized soil with neutral inoculum and inoculum of co-selected soil biota from original plots) and **(c)** native soil (unsterilized soil with co-selected soil biota from original plots). Raw means and standard errors are shown (for significances see Table S1b).

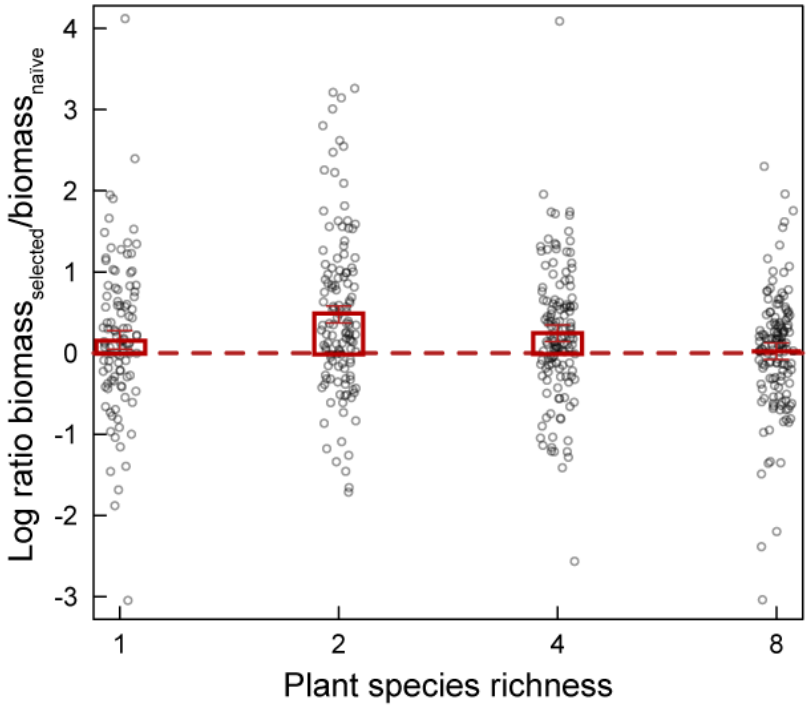

Figure 3 Log ratio of productivity in communities of co-selected plants ($bm_{selected}$) and productivity in naïve communities ($bm_{naïve}$) across years and soil treatments. In 8-species mixtures, productivity did not differ between communities of co-selected and naïve plants (ratio=0). Especially in 2- and 4-species mixtures, but also in monocultures, communities of co-selected plants produced more biomass than naïve communities. Means and standard errors are shown. Raw data are plotted in the background.

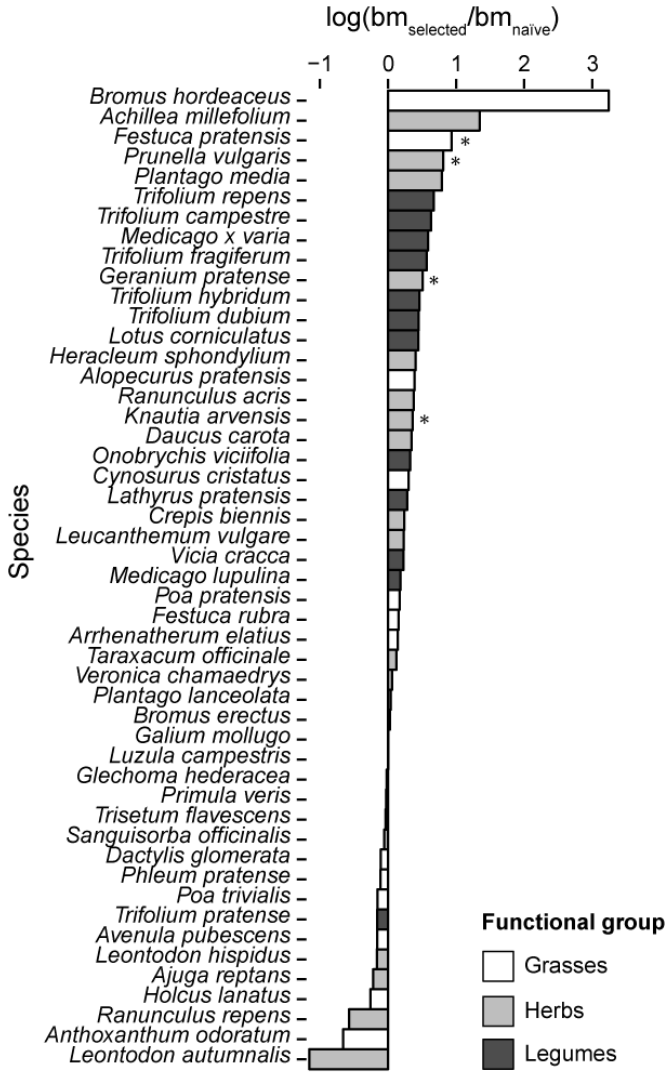

Figure 4 Log-transformed species biomass ratios between co-selected and naïve plants. The majority of plant species attained greater aboveground biomass in communities of co-selected plants compared with naïve communities. The studied plant species belong to three different functional groups: grasses (white bars), herbs (light grey bars) and legumes (dark grey bars). Data are for each species across the four experimental years, across soil treatments and across species richness levels and species compositions of communities (n = 32–352). Three species with n < 32 were excluded from the analysis (*Anthriscus sylvestris*, *Campanula patula* and *Cardamine pratensis*). The stars represent P-values < 0.05 for species tested separately.

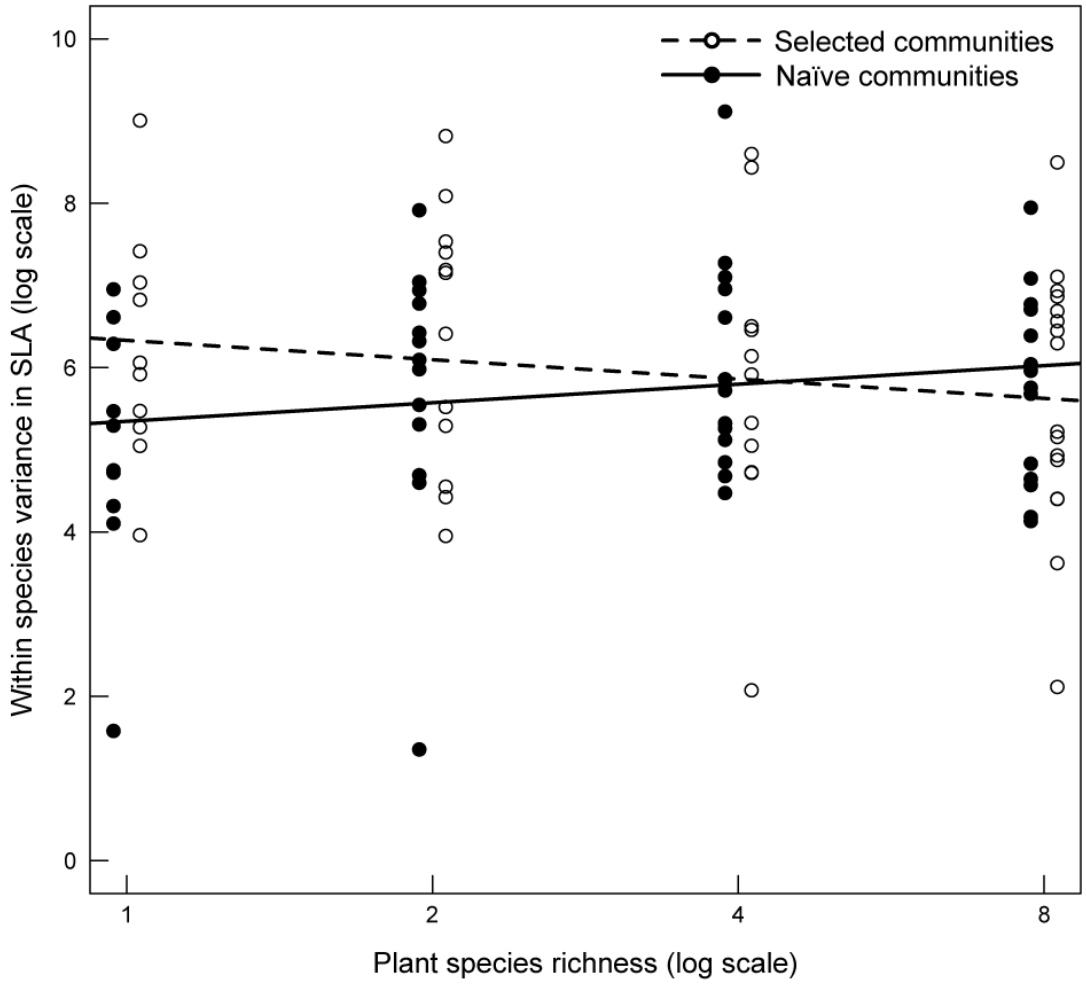

Figure 5 Within-species variation in specific leaf area (SLA) for communities of co-selected plants and naïve communities at the end of the experiment in 2015 in neutral soil. In monocultures within-species variation in SLA (measured as the within-species variance component in analysis of variance) was greater for co-selected than for naïve plants and this difference decreased with increasing species richness. Open circles and dashed line refer to communities of co-selected plants, closed circles and solid line refer to naïve communities. The interaction of log(species richness) and plant history was significant ($F_{1,69.2} = 4.87$, $P = 0.031$).

Supporting Information

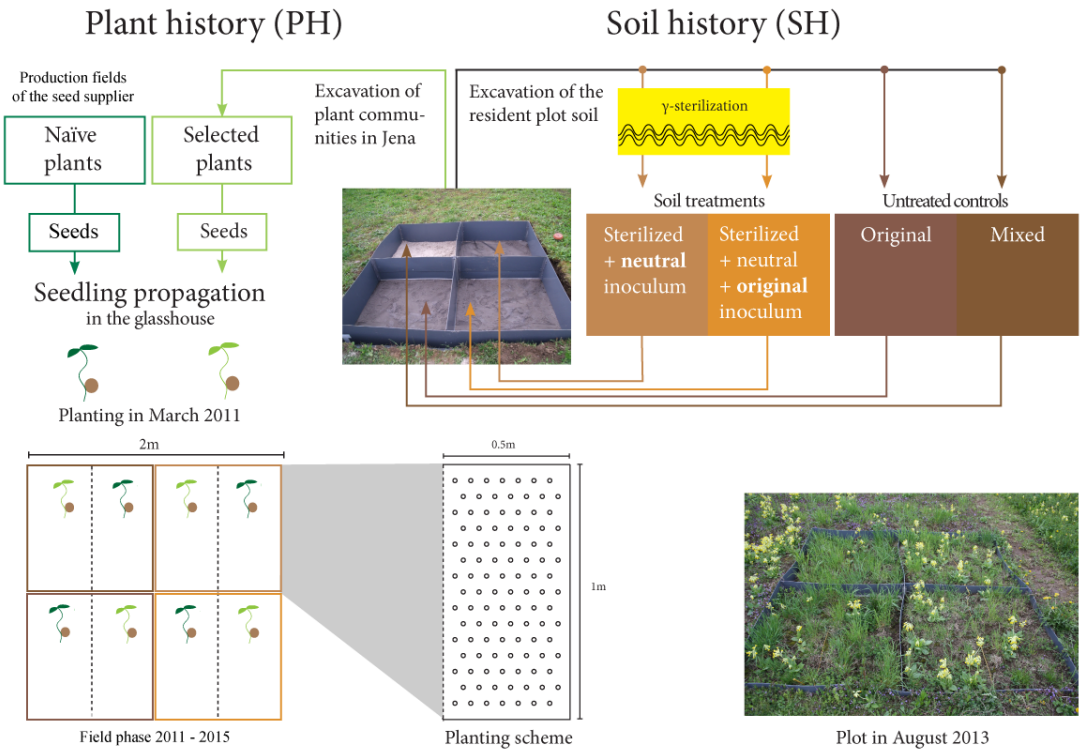

Figure S1 Experimental design. In a glasshouse, co-selected plants were propagated from seeds of plants, which were previously excavated from their communities in the experimental field; naïve plants were propagated from seeds purchased from a seed supplier. Subsequently, the seedlings were planted in the field according to randomized planting schemes with equal species densities. Communities of co-selected plants (light green) and of naïve plants (dark green) were grown in four different soil treatments filled into quadrats (shades of brown), either sterilized or unsterilized, and either containing native soil (with co-selected soil biota) or not. One of the four soil treatments (mixed soil) was forgone after two years of the experiment because the plants were used for a different experiment. Data from this fourth treatment were therefore excluded from all analyses presented in this paper.

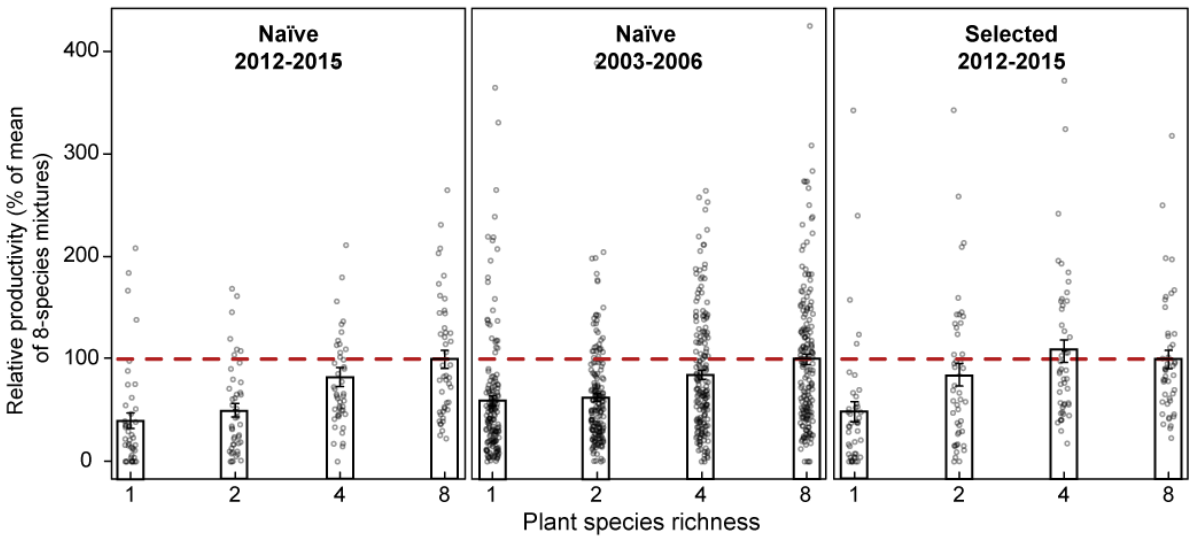

Figure S2 Relative productivity (% of mean 8-species mixture) of naïve plant communities in the current experiment and at the beginning of the Jena Experiment and of communities of co-selected plants, which had been derived from the second type after 8 years of community evolution. The two types of naïve plant communities had similar productivity but were significantly different from the communities of co-selected plants. Means and standard errors of treatments with neutral soil are shown. 100% is indicated by dashed line.

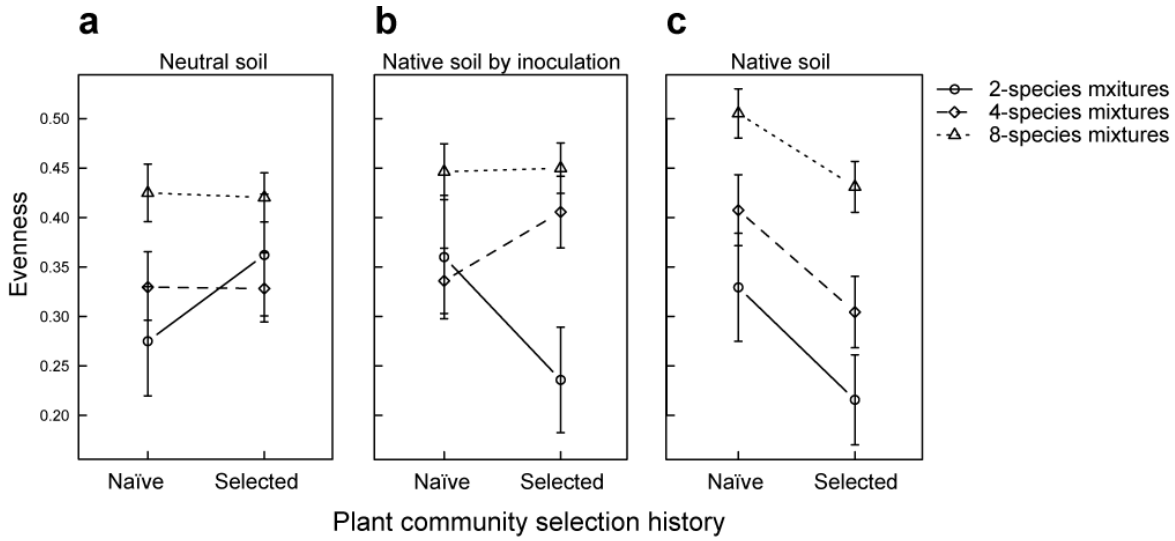

Figure S3 Evenness of naïve communities and communities of co-selected plant species. Evenness was slightly increased in naïve plant communities across all soil treatments ($F = 4.088$, $P = 0.046$ for main effect of plant history), which was driven by a much higher evenness for naïve communities on native soil. a, Evenness of selected and naïve plant communities on neutral soil obtained by sterilization and inoculation ($F = 1.593$ and $P = 0.209$ for effect of plant history). b, Evenness of selected and naïve plant communities in native soil obtained by inoculation (inoculum of co-selected microbial communities) ($F = 0.360$ and $P = 0.55$ for effect of plant history). c, Evenness of selected and naïve plant communities in native soil containing co-selected microbial communities ($F = 20.10$ and $P < 0.001$ for effect of plant history). Means and standard errors are shown.

Table S1 Results of mixed-effects ANOVA for the aboveground biomass of the test communities. **(a)** Productivity and **(b)** relative productivity (% of mean productivity of 8-species mixtures per plant history-by-soil treatment-by-year combination).

a	**Productivity**			
Source of variation	**nDf**	**dDF**	***F***	***P***
Factorial species richness (facSR)	3	42.2	7.81	**< 0.001**
Soil history (SH)	2	218.8	16.68	**< 0.001**
facSR × SH	6	218.7	0.64	0.697
Plant history (PH)	1	191.3	23.87	**< 0.001**
facSR × PH	3	191.2	2.77	**0.043**
Factorial harvest (Har)	3	121	1.26	0.290
facSR × Har	9	121	0.99	0.451
SH × Har	6	263.2	4.07	**0.001**
facSR × SH × Har	18	263.2	2.44	**0.001**
PH × Har	3	398.9	2.44	0.064
Variance components	**n**	**Var**	**SE**	**z-ratio**
Plot	47	3707.2	1252.2	2.96
Quadrat	141	0.0	0.0	na
Plot × Har	188	3736.8	762.0	4.90
Half-quadrat	282	2678.8	530.5	5.05
Quadrat × Har	564	1495.1	611.3	2.45
Residual	1128	9046.0	645.7	14.01

b	**Relative productivity**			
Source of variation	**nDf**	**dDF**	***F***	***P***
Factorial species richness (facSR)	3	42.2	8.04	**< 0.001**
Soil history (SH)	2	213.6	16.48	**< 0.001**
facSR × SH	6	213.5	0.53	0.789
Plant history (PH)	1	192.1	16.75	**< 0.001**
facSR × PH	3	191.9	2.90	**0.036**
Factorial harvest (Har)	3	121.1	0.54	0.656
facSR × Har	9	121.1	1.05	0.402
SH × Har	6	263.2	3.91	**0.001**
facSR × SH × Har	18	263.2	2.01	**0.010**
PH × Har	3	401.1	4.23	**0.006**
Variance components	**n**	**Var**	**SE**	**z-ratio**
Plot	47	720.4	240.8	2.99
Quadrat	141	0.0	0.0	na
Plot × Har	188	703.5	144.3	4.87
Half-quadrat	282	496.4	101.7	4.88
Quadrat × Har	564	250.0	117.9	2.12
Residual	1128	1803.1	128.4	14.05

Note: nDF = numerator degrees of freedom, dDF = denominator degrees of freedom, *F* = variance ratio, *P* = probability of type-I error. Number of replicates (n), variance components (Var) and associated standard errors (SE) for the random effects are provided.

"Factorial species richness" refers to the four diversity levels 1, 2, 4 and 8; "plant history" refers to the community-evolution treatment comparing naïve communities with communities of co-selected plants; "soil history" refers to the three soil treatments and "factorial harvest"

Table S2 Results of mixed-effects ANOVA for the evenness of selected and naïve plant communities.

	Response: Evenness			
Source of variation	**nDf**	**dDF**	***F***	***P***
Factorial species richness (facSR)	2	33.5	3.74	**0.034**
Soil history (SH)	2	140.4	0.34	0.715
facSR × SH	4	140.4	1.25	0.292
Plant history (PH)	1	132.2	4.28	**0.041**
facSR × PH	2	132.2	0.68	0.508
SH × PH	2	132.2	6.84	**0.001**
facSR × SH × PH	4	132.2	3.43	**0.010**
factorial harvest (Har)	3	99.3	7.29	**< 0.001**
Variance components	**n**	**Var**	**SE**	**z-ratio**
Plot	47	1.00E-02	4.58E-03	2.191
Quadrat	141	4.04E-09	3.23E-10	na
Plot × Har	188	2.28E-02	4.29E-03	5.317
Half-quadrat	282	1.63E-03	1.37E-03	1.189
Quadrat × Har	564	3.98E-03	2.21E-03	1.801
Residual	1128	3.31E-02	2.65E-03	12.486

Note: nDF = numerator degrees of freedom, dDF = denominator degrees of freedom, *F* = variance ratio, *P* = probability of type-I error. Number of replicates (n), variance components (Var) and associated standard errors (SE) for the random effects are provided.

"Factorial species richness" refers to the four diversity levels 1, 2, 4 and 8; "plant history" refers to the community-evolution treatment comparing naïve communities with communities of co-selected plants; "soil history" refers to the three soil treatments and "factorial harvest" refers to the four years 2012–2015.

Table S3 Analysis of similarity (anosim) results for the pairwise comparison of three soil treatments.

Year	Enzyme	Soil comparison	*R*	*P*
2011	Hh	Native vs Neutral	0.307	0.002
2011	Hh	Native vs Native by inoculation	0.258	0.002
2011	Hh	Neutral vs Native by inoculation	0.501	0.472
2011	Taq	Native vs Neutral	0.443	0.002
2011	Taq	Native vs Native by inoculation	0.389	0.002
2011	Taq	Neutral vs Native by inoculation	0.258	0.042
2012	Hh	Native vs Neutral	0.698	0.002
2012	Hh	Native vs Native by inoculation	0.586	0.002
2012	Hh	Neutral vs Native by inoculation	0.389	0.002
2012	Taq	Native vs Neutral	0.627	0.002
2012	Taq	Native vs Native by inoculation	0.501	0.002
2012	Taq	Neutral vs Native by inoculation	0.586	0.006

Note: R = statistic R-value, *P* = significance, number of permutations is 505, calculated with Bray-Curtis dissimilarity. Native soil contained co-selected microbial communities.

CHAPTER TWO

Community evolution increases community stability in a grassland biodiversity experiment

Community evolution increases community stability in a grassland biodiversity experiment

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Author contributions

B.S. conceptualized the project; S.J.V.M. and T.H. carried out the experiment; B.S., C.W. and S.J.V.M. analysed the data; S.J.V.M. and C.W. wrote the first draft of the manuscript. All authors contributed to the final manuscript.

ABSTRACT

The recent increase in extreme weather events demands a deeper understanding of how communities respond to environmental perturbations. It is known that biodiversity increases stability in grassland plant communities during unperturbed states and in response to environmental perturbations such as droughts and floods. We hypothesized that not only plant diversity but also community selection history can buffer the impact of perturbations on plant communities and consequently increase ecosystem stability both during perturbed and unperturbed states. Using a long-term biodiversity experiment with 52 species growing in four species diversity levels, we tested both the influence of plant and soil community history on the stability of plant community productivity over four years, and on resistance, resilience and recovery in response to a flood. We grew selected plant communities with eight years of co-occurrence in the field adjacent to identical but naïve communities lacking such a common history. The communities were planted in native soil, neutral soil and sterilized soil containing a native soil inoculum. Selected plant communities were more stable over time, especially in low diversity plots, and recovered better from the flooding event. Native soil treatments did stabilize productivity during unperturbed states but did not further increase resistance to the flood. We reconfirmed the importance of biodiversity for ecosystem stability and for the first time showed that community evolution has an even stronger buffering effect. Our results suggest that community-level evolutionary processes might play an important role in mediating ecosystem stability, which has implications on ecosystem management and conservation strategies.

Keywords: community selection history | compensatory dynamics | environmental perturbation | flood | grassland biodiversity | recovery | resilience | resistance | stability

SIGNIFICANCE STATEMENT

In a changing world, it is crucial to understand the mechanisms maintaining ecosystem stability. Here we tested whether selected plant communities consisting of plants that had been growing together for almost a decade were more stable than naïve communities consisting of plants without a history of co-occurrence. We grew selected and naïve communities in native and in neutral soil and assessed stability during unperturbed states and in response to a flood. Biodiversity and, more importantly, evolutionary processes within and between plant species buffered our experimental communities. This suggests that we need to protect interacting species within their community context and not in isolation, if we seek to preserve well-functioning communities and ecosystems.

Introduction

With climate change, extreme weather events such as storms, droughts or floods are increasing in both frequency and severity (1, 2), urging the need to understand how ecosystems cope with these perturbations. It has long been recognized that biodiversity can provide greater stability to ecosystem functioning (3, 4) by buffering the impact of environmental perturbations (5). The relationship between plant diversity and the temporal stability of ecosystem productivity through improved resistance, resilience and recovery has been studied extensively (6–9). Specifically, there is growing evidence that shows ecosystem resistance and resilience depend on species richness (8, 9), plant density (10), and plant functional traits (11).

Plant communities may be relatively unaffected by an extreme climate event via a range of different mechanisms. First, plant communities could resist change in functioning when faced with a perturbation. In other words, communities have the capacity to withstand environmental perturbations by absorbing the impact. This resistance to perturbations is an important component of ecosystem stability (12). Secondly, despite a reduction in functioning during the event, communities could exhibit resilience by rapidly recovering to the former state after the perturbation (13, 14). Though these three measures of stability are all inherently linked, they can also be analyzed and tested independently to pin down possible mechanisms of stability in the face of perturbations. Compensatory mechanisms have been suggested to facilitate stability of productivity in grasslands ecosystems (15–18). For example, asynchronous fluctuations among taxa at the population level may result in the maintenance of the overall community performance because the decline in the performance of some species are compensated by other community members such that the overall performance of the community is maintained (15–17). Therefore, more diverse communities can enhance the stability of the community because there is a higher probability that some species will maintain the performance of the community within a changing environment; often referred to as the insurance or portfolio effect (15, 17, 19, 20). Such asynchronous patterns in the temporal performance of a population can be quantified and assessed as potential mechanisms behind the stability in the net performance of a community (17, 21, 22).

There is growing evidence that community evolution may influence ecosystem performance, but this evidence stems mostly from microbial microcosm experiments (23–25). Hence, there is a need for evidence to indicate whether co-evolved communities in more natural settings can provide greater ecosystem stability through greater resistance and recovery under environmental perturbations. Community evolution was defined as “a genetically based change in the ecological interactions that occur between species over time“ (26). Although it is conceivable that community evolution is the norm in communities comprised of several interacting populations, the importance of ecologically relevant short-term evolution for community stability is entirely unknown (27). So far, evolutionary mechanisms underlying the diversity–stability relationship have focused on the effect of phylogenetic relatedness on stability that reflects evolutionary mechanisms over broad

time scales (28, 29). It remains unclear though, how a selection history of co-existence on a shorter time scale can influence the stability of communities in response to extreme climatic events.

The functioning of plant communities is also inherently determined by the soils in which they establish, and plant communities in turn influence the biotic and abiotic soil characteristics. For instance, the presence, diversity and composition of soil biota can have a positive influence on plant community productivity (30). More importantly, the importance of soil community composition and diversity has also been found to contribute to ecosystem stability in grasslands (31–33). Compared to grassland plant communities, soil microbial communities generally exhibit a shorter generation time and faster turnover likely allowing for microbial community co-evolution with their associated plant communities to influence plant community stability (34–36).

Here we assess the role of community evolution on the stability of productivity when faced with a major flooding perturbation in a long-term grassland biodiversity experiment in Central Europe (The Jena Experiment). In June 2013, Central Europe experienced a flood affecting our test site (37) and covering the experimental field site in maximum 0.4 m depth of water for over two weeks. Floods alter ecosystem productivity (38) and plant community properties such as diversity or composition likely play a pivotal role in an ecosystems resistance to, and recovery from flooding. Hence, this flood posed a unique opportunity to study the role of plant diversity and community composition on buffering against the impact of flooding on ecosystem functioning.

Stability can be viewed in two main ways: in terms of long-term temporal variability of productivity and in shorter-term resistance and resilience to environmental perturbations (7, 10). In this study, we assessed ecosystem temporal stability of plant productivity as well as resistance, recovery and consequently resilience of our test communities to this flood. Specifically, we test the hypothesis that plant communities consisting of species that co-occurred over a longer period of time may be co-adapted to be more stable relative to communities consisting of plants without a common selection history. Consequently, we expect that community evolution may increase temporal ecosystem stability during unperturbed states (hypothesis 1) as well as resistance, recovery and resilience in response to a flooding event (hypothesis 2). Incorporating also the importance of the soil environment, we anticipated that a common selective past with the soil biota would further increase stability during both perturbed and unperturbed states (hypothesis 3).

To address our hypotheses, we conducted an experiment within a long-term biodiversity experiment running since 2002 in Jena, Germany (Jena Experiment, see (39) and compared plant communities selected for eight years to naïve plant communities without such a shared selection history. We used experimental plant communities comprised of 1, 2, 4 or 8 species with 12 unique species compositions within each species richness level and measured community-level plant productivity each spring and summer from 2012 to 2015 by collecting species-specific

aboveground biomass (see Materials and Methods). To connect above- and belowground interactions, we factorially combined two community components under selection: plants and soils. We grew selected and naïve plant communities for four years in native soil with a common selection history with the selected plant communities, or in neutral soil without a common selection history, with either selected or naïve plant communities (hypothesis 3).

Results

Community productivity. Species richness increased community productivity in both selected and naïve plant communities (Fig.1). Overall biomass was double in the eight species mixtures compared to monocultures. Aboveground community biomass was also greater on average for selected plant communities in monocultures, 2- and 4-species mixtures, but not in 8-species mixtures (Fig. 1). Mean community biomass was halved by the flood for both selected and naïve plant communities in monocultures, 4-and 8-species mixtures (Fig.1). The 2-species mixtures experienced a less dramatic biomass loss from flooding.

The soil treatments significantly influenced community biomass and its variation over time. Soil sterilization increased community biomass during the first three harvests before the flood (May 2015, August 2012 and May 2013) compared to unsterilized native soils, but this effect was strongly reduced post-flood (*SI Appendix* Fig. S2*A*). During the first three harvests, the soil treatment had a large effect especially in 4- and 8-species mixtures (*SI Appendix* Fig. S2*A*). In 8-species mixtures, the neutral soil (sterilized soil with a neutral inoculum) more than doubled community productivity when compared to soil treatments including a native inoculum (*SI Appendix* Fig S2*A*). During the three harvests post-flood (May 2014, August 2014 and May 2015), soil treatments did not significantly affect plant community productivity in 2-and 8-species mixtures. In monocultures and 4-species mixtures, neutral soil continued to have a positive influence on productivity (*SI Appendix* Fig. S2*A*).

Temporal stability during unperturbed states (hypothesis 1). Species richness significantly increased stability for both selected and naïve communities both pre- and post-flood (Table 1). Species richness also increased stability across the entire time of the experiment, but less so for selected communities (Fig. 2, Table 1, interaction ”logSR” with “plant history”). During the unperturbed pre-flood conditions, selected plant communities were significantly more stable than naïve plant communities in the 2-species mixtures (Fig. 2, Table 1). In the other species richness levels the stability of naïve plant communities did not differ significantly from selected plant communities. In contrast, stability post-flood was greater in the selected plant communities overall (Fig. 2, Table 1), and significantly so in 2- and 4-species mixtures. The community-evolution treatment did not affect overall stability across the entire time period (Table 1, main effect “plant history”).

Resistance, recovery and resilience of selected and naïve plant communities (hypotheses 2 and 3). Recovery was significantly greater in selected plant communities (Fig. 3*AC*, Table 2 and *SI Appendix* Table S1). For resistance and resilience, however, we did not find a significant difference between naïve and selected plant communities (Fig. 3*ABD*, Table 2 and *SI Appendix* Table S1). For recovery and resistance, we observed a significant interaction between the community-evolution treatments and species richness (Table 3, interaction "logSR" with "plant history"). In monocultures, resistance and recovery (and also resilience) were very similar between naïve and selected communities. In contrast, at high diversity (4- and 8-species mixtures) naïve communities were more resistant (Fig. 3*B*), but recovered worse (Fig 3*C*) than selected communities.

The three soil treatments significantly influenced resistance and resilience (Table 2, *SI Appendix*, Fig. S2*B* and Table S1). Plant communities in native soil had the lowest community biomass pre-flood and hence also exhibited a lower biomass reduction in response to the flood, resulting in higher resistance (*SI Appendix* Fig. S2*B*). The low biomass pre-flood also resulted in a high resilience, where plant communities were more productive relative to themselves post-flood compared to pre-flood in native soil. Plant communities in sterilized soil with a neutral inoculum experienced a dramatic reduction in biomass especially in the 4- and 8-species mixtures (*SI Appendix* Fig. S2*B*).

Population-level mechanisms underlying stability. Stability was negatively related to population synchrony for selected and naïve communities (Fig. 4*A*, Table 3). Paralleling this, community-level coefficient of variance (inverse of stability) was generally positively related to population synchrony, but more so for naïve communities (Fig. 4*D*, Table 3). Species synchrony was also negatively related to population-level variance, but the naïve communities showed a stronger response (Fig. 4*C*, Table 3). Species richness interacted with the response of the different plant communities to population-level variance. The selected communities showed a lower population-level variance at low species diversity, whereas at high diversity the trend was reversed. Stability was negatively influenced by population-level variance for naïve and selected plant communities and (Fig. 4*B*, Table 3). The community-evolution treatments did not influence synchrony directly.

Influence of compositional changes in selected and naïve plant communities. Species richness had the greatest influence on composition turnover and significantly increased pre-flood turnover (Fig. 5, $F_{1,33.9} = 6.749$, $P = 0.014$, *SI Appendix* Table S2). There was no overall effect of the community-evolution treatment on turnover between selected and naïve communities (Fig. 5, $F_{1,102.9} = 0.98$, $P = 0.325$ for the main effect "Plant history", *SI Appendix* Table S2). However, pre-flood turnover was higher for selected plant communities at high diversity and lower for selected communities at low diversity (Fig. 5*C*, $F_{1,102} = 5.18$, $P = 0.025$ for the interaction between plant history and species richness, *SI Appendix* Table S2).

A species-level analysis of resistance, recovery and resilience revealed that neither functional group nor species identity was driving the observed community-level effects (*SI Appendix* Fig. S4). We calculated the log-ratio between relative resistance, recovery and resilience between selected and naïve communities and found that for each stability measure, approximately half of the species contributed to a greater or lesser stability, respectively (*SI Appendix* Fig. S4).

Influence of mean pre-flood community productivity on resistance, recovery and resilience. The productivity pre-flood (mean of the three harvests before the flood) had a significantly negative effect on absolute resistance ($F_{1,240.2} = 27.7$, $P < 0.001$), as well as the relative resistance and ($F_{1,244.1} = 275.2$, $P < 0.001$, *SI Appendix* Fig. S3). In contrast, relative recovery was not affected by the community productivity ($F_{1,245.7} = 0.103$, $P = 0.749$, *SI Appendix* Fig. S3). However, the more productive communities pre-flood exhibited lower absolute resilience ($F_{1,229.2} = 60.97$, $P < 0.001$), as well as relative resilience ($F_{1,233.5} = 124.6$, $P < 0.001$, *SI Appendix* Fig. S3). Absolute recovery was positively related with mean pre-flood community productivity ($F_{1,222.5} = 10.36$, $P = 0.001$, *SI Appendix* Fig. S3).

Discussion

Previously we found that community evolution increased community productivity in diversity levels up to four species (40). Building on these earlier findings we anticipated that eco-evolutionary processes among plant species and between plants and their soils could furthermore increase community stability. We found that selected plant communities were more stable over time following their recovery after experiencing flooding. This provides some support for our hypothesis that selected plant communities would be more stable over time during unperturbed states (hypothesis 1). In support of our second hypothesis that selected communities would exhibit greater stability over the flooding perturbation due to greater resistance, recovery and resilience, we found that selected plant communities recovered better from flooding compared to naïve plant communities. Naïve plant communities also recovered, but not to the same extent as selected communities. Previously it was shown that complementarity can increase due to community evolution (39). Our results that community evolution can also result in greater recovery and resilience from an extreme climate event may further reflect species facilitative effects or possibly the density-dependence of resilience (10).

Selected plant communities did not exhibit greater resistance and resilience to the flooding. Our finding that diversity enhanced recovery, but not resistance, parallels those in a similar grassland diversity gradient in response to drought (41). In our communities, we found that lower resistance and resilience was due to greater pre-flood productivity. In this sense, selected communities had “more to lose” when faced with this extreme climate event, an observation reported also in other grassland systems in response to drought (10, 42). For instance, Wang et al. (2009)

demonstrated that communities with lower biomass were more resistant to drought in terms of absolute losses in productivity (42). Further a previous study in the same grassland system also found that species richness reduced community resistance in response to the flood, because species-rich communities had greater productivity after the flood (11). Thus, overall the reduced productivity of naïve communities resulted in less absolute loss in productivity due to the flood and their greater resistance. It was previously shown that selection for niche differentiation in results in higher community productivity (43). Here our results indicate that this selection driven increase in productivity may consequently reduce the resistance to extreme climate events.

Compensatory dynamics are known to increase ecosystem stability and maintain ecosystem function through environmental perturbation (16). Here we assessed species synchrony and population-level variance as the underpinning mechanisms behind the stability in selected and naïve communities. As anticipated, species synchrony decreased stability. However, the community-evolution treatment did not directly influence synchrony. Instead, community evolution influenced the relationship between species synchrony and community-level variance. Intriguingly, where synchrony was high selected communities exhibited greater stability, but where synchrony was low selected and naïve plant communities were similarly stable. This suggests that in selected plant communities, species varied similarly through time, but the variations were relatively smaller. Synchrony was strongly negatively related to population-level variance. In other words, communities with species that varied more through time also exhibited lower synchrony indicating that species differed in performance at different times. The more negative relationship in the selected communities suggests that these communities exhibited greater compensatory dynamics. Taken together, our results show that selected plant communities maintained a more stable productivity following flooding via greater compensatory dynamics at the population-level.

It is important to note, that the increase in stability in our study was not due to compositional changes. Our analysis of composition turnover revealed that selected and naïve plant communities on average did not differ in their compositional changes. Only, when turnover interacted with diversity did we find that selected plant communities had lower turnover at low diversity, and greater turnover at high diversity. We can also rule out that few species are driving the overall effects. Analyzing resistance, recovery and resilience for each species separately revealed that about half of the species were more resistant in selected communities and the other half more resistant in naïve communities (*SI Appendix* Fig. S4*A*). A similar pattern was observed for recovery and resilience (*SI Appendix* Fig. S4*BC*); and also functional group identity did not determine whether a species was more or less resistant.

The interactions between plants and their soil communities are well known to influence ecosystems functioning (44). We therefore anticipated that soils would play a significant role in the resistance, recovery and resilience in plant community

productivity when facing environmental perturbation (hypothesis 3). Surprisingly, we did not find any evidence for an interaction between soil treatment and plant community selection history. Thus, all plant community-evolution effects were independent of soil treatment. Nevertheless, as expected, we did find some direct effects of soil treatments on aboveground biomass. Plant communities established in their native soil were generally less productive pre-flood. Consequently, the resistance was greater in native soil because of the lower pre-flood productivity. The lower pre-flood productivity in native soil may indicate i) that there was a greater density of antagonistic biotic interactions with the soil community and ii) that the nutrient pool of these soils may have been drawn down by the plant community previously, compared to an initially sterilized standard inoculated soil, or iii) the greater pre-flood productivity in inoculated soils may have resulted from a nutrient flush following the initial soil sterilization (45). Nonetheless, the difference between inoculated and native soils was not observed post-flood hinting that the flooding may have equilibrated the soil properties among soil treatments. More intriguingly, due to the greater post-flood productivity compared to the pre-flood productivity, plants grown in native soils generally exhibited greater resilience to the flood. These results complement findings from a study by Lau and Lennon (2012), who found that plants faced with environmental stress benefited from association with soil microbial communities that adapted faster to neutral conditions.

Our study is the first to demonstrate the importance of evolutionary processes among plant species in maintaining a stable productivity in grasslands through improving ecosystem recovery and resilience. Maintaining ecosystem resilience has been suggested to be crucial for conservation purposes and ecosystem management (46), which calls for a deep understanding of the mechanisms facilitating stability. Here we report that community evolution of plant species comprising a plant community can increase stability and recovery of such ecosystems. These results have large implications for ecosystem management and conservation practices.

Materials and Methods

Field site. This study was conducted at the Jena Experiment field site (Jena, Thuringia, Germany, 51˚N, 11˚E, 135 m a.s.l.) from 2011 to 2015. The Jena Experiment is a long-term biodiversity field experiment located on the banks of the Saale River. In 78 experimental field plots of different diversity levels, 60 central European grassland species are grown in a number of species combinations since 2002 (39).

Community-evolution treatments. The study included eleven monocultures, twelve 2-species mixtures, twelve 4-species mixtures and twelve 8-species mixtures for a total of 47 experimental plots. We used two community-evolution treatments; plants with eight years of shared community selection in these experimental plots (selected communities) and plants without a common selection history in the Jena Experiment (naïve communities). The naïve plant seeds without a common selection history were obtained from the same commercial seed supplier (Rieger Hofmann GmbH, in

Blaufelden-Raboldshausen, Germany); the supplier for seeds used for the establishment of the original Jena Experiment plant communities (38). The supplied seeds were collected originated from various field sites in Germany and have been cultivated by reseeding every year for at least five years in monoculture. Seeds of selected communities were produced in an experimental garden in Zurich, Switzerland, from cuttings that had been made in the Jena Experiment. The cuttings were planted in Zurich in the original species combination in plots fenced with plastic netting to reduce pollination between communities (43). A small number of seeds were additionally collected directly in the plots of the Jena Experiment. The "selected" seeds were thus offspring of plant populations that had been sown in 2002 and grown until 2010 in plots of the Jena Experiment.

In January 2011, the seeds of selected and naïve communities were germinated in potting soil (BF4, De Baat; Holland) in a glasshouse in Zurich. Subsequently, the seedlings were transported back to the Jena Experiment field site and transplanted into 2 x 2 m subplots of the original plots (in March 2011). There were four 1 x 1 m quadrats with different soil treatments in each subplot (see next section) and each quadrat was split into two 1 x 0.5 m halves ("half-quadrats"). We planted seedlings of selected communities into one half and seedlings of naïve communities into the other half of each quadrat in a hexagonal pattern at a density of 210 plants per m^2 with a 6-cm distance between individuals. We planted the species in equal proportions, but five species were excluded from both selected and naïve communities because they were no longer present in the original plot of the Jena Experiment. After transplanting, the seedlings received water every second day for six weeks.

Soil treatments. Within each 2 x 2 m subplot of the 47 plots of the Jena Experiment, we removed the original plant cover in September 2010 and used it for the plant propagation in the experimental garden in Zurich (see previous section). Subsequently, we excavated the soil to a depth of 0.35 m, added a 10-cm layer of sand to the bottom of the plots and covered it with a 0.5 mm mesh net. The borders of the quadrats and the subplots we separated by plastic frames. The excavated native soil from each of the plots was sieved and four soil treatments were prepared. Half of the soil (approximately 600 kg per plot) was gamma-irradiated to remove the original soil biota. Half of this sterilized soil was then inoculated with 4 % (by weight) of live sugar-beet soil and 4 % of sterilized native soil of the corresponding plot ("neutral soil" obtained by inoculation). Live sugar-beet soil was added to create a neutral soil community and was previously collected in an agricultural sugar-beet field not associated with the Jena Experiment, but with comparable soil properties. The second half of the gamma-irradiated soil was inoculated with 4 % (by weight) of live sugar-beet soil and 4 % of live native soil of the corresponding plot ("native soil" obtained by inoculation). The non-sterilized part of the excavated soil was used for the second two soil treatments. Half of this soil was filled back into one quadrat of the corresponding plot ("native soil"). The other half of the unsterilized soil was mixed among all plots and filled into the remaining quadrats. However, this fourth soil

treatment was abandoned after two years, which is why this treatment is not included in the present study.

The soils were left to rest in closed bags to allow for the soil chemistry to equalize and to encourage soil biota of the inocula to colonize the sterilized soil before planting. The soils were then added into the quadrats in December 2010 and all quadrats were covered with a net and a water permeable black sheet to avoid spilling between quadrats until the seedling transplantation in March 2011.

Sampling of aboveground biomass. The test communities were weeded three times a year and the plants were cut to three cm aboveground twice a year at typical grassland harvest times (late May and August) in central Europe. The harvested plant material from a 50 x 20 cm area in the centre of each half-quadrat was collected to measure aboveground biomass. We sorted the plant material into species, dried it at 70°C and weighed the dried biomass.

Natural flooding event. In June 2013, the field site was flooded due to heavy rains in central Europe (37, 47). The flood duration (maximum 12 days) and depth of water (maximum of 40 cm) was variable among plots and quadrats due to small topographical differences among the plots in the experiment (14). The variation in flooding severity was distributed across the diversity gradient and within the plots, the half-quadrats experienced the same flooding severity.

Data analysis. For the present study, we analysed the data from seven harvests (spring/summer 2012, spring/summer 2013, spring/summer 2014 and spring 2015). We calculated ecosystem temporal stability by dividing the mean community biomass for a time period by the standard deviation of the same interval (3, 8, 22). To characterize pre-flood stability, we used the three harvests from May 2012, August 2012 and May 2013, and to characterize post-flood stability, we used the three harvests May 2014, August 2014 and May 2015. Hence, both time periods consisted of two spring harvests and one summer harvest. In addition, we calculated temporal stability for the entire duration of the experiment.

In addition to temporal stability, we calculated relative resistance in response to the flooding event as the log of the productivity ratio during flood/pre-flood, using the mean productivity of the three pre-flood harvests as the denominator. Similarly, we calculated relative recovery as the log of the productivity ratio post-flood/during flood, using the mean of the three post-flood harvests as the nominator. Finally, we calculated relative resilience as the log of the productivity ratio post-flood/pre-flood, using the means of the corresponding three harvests for nominator and denominator. The results using log-ratios were compared with those using differences; we refer to these as absolute resistance, absolute recovery and absolute resilience. Fig. 3 visualizes these calculations.

For population-level analyses we calculated species synchrony (21, 48) and population variance (weighted by the community net productivity see (17), where popCV * sqrt(Sync) = CV). Compositional turnover (Bray-Curtis dissimilarity) we calculated using the vegdist() function from the R package vegan (49). Using a linear regression, we then compared how these measures relate to each other.

To test the influence of plant and soil community history on stability, resistance, recovery and resilience, we fitted linear mixed-effects models with these response variables and summarized results in analyses of variance (ANOVA) tables. Significance tests were based on approximate F-tests using appropriate error terms and denominator degrees of freedom (50). The fixed-effects terms in the model were factorial species richness of the original plots of the Jena Experiment, year and season of the harvest, soil treatments (native, sterilized + native inoculum and sterilized + neutral inoculum), plant community-evolution treatment (naïve vs. selected communities) and interactions of these. The random-effects terms were plot, quadrat, half-quadrat and, if applicable, their interactions with time. Statistical analyses were conducted using the software product R, version 3.2.3 (R Core Team 2015). Mixed models using residual maximum likelihood (REML) were fitted using the package ASReml for R (51) and Asreml plus from the package 'Pascal' available at github (50).

ACKNOWLEDGMENTS

Thanks to Debra Zuppinger-Dingley, Dan Flynn and Varuna Yadav for the establishment of the experimental plots. Thanks to the Jena Experiment for providing infrastructure and help and to D. Trujillo and M. Furler for technical assistance. This study was supported by the Swiss National Science Foundation (grant numbers 130720, 147092 and 166457 to B.S.) and the University Research Priority Program Global Change and Biodiversity of the University of Zurich. The Jena Experiment is funded by The Deutsche Forschungsgemeinschaft (DFG, German Research Foundation, FOR1451).

AUTHOR CONTRIBUTIONS

B.S. designed research, T.H. and S.J.V.M. performed research; S.J.V.M., C.W. and B.S. analyzed data; S.J.V.M. wrote the paper with substantial contribution of the other authors.

The authors declare no conflicts of interest.

Supporting Information is available online.

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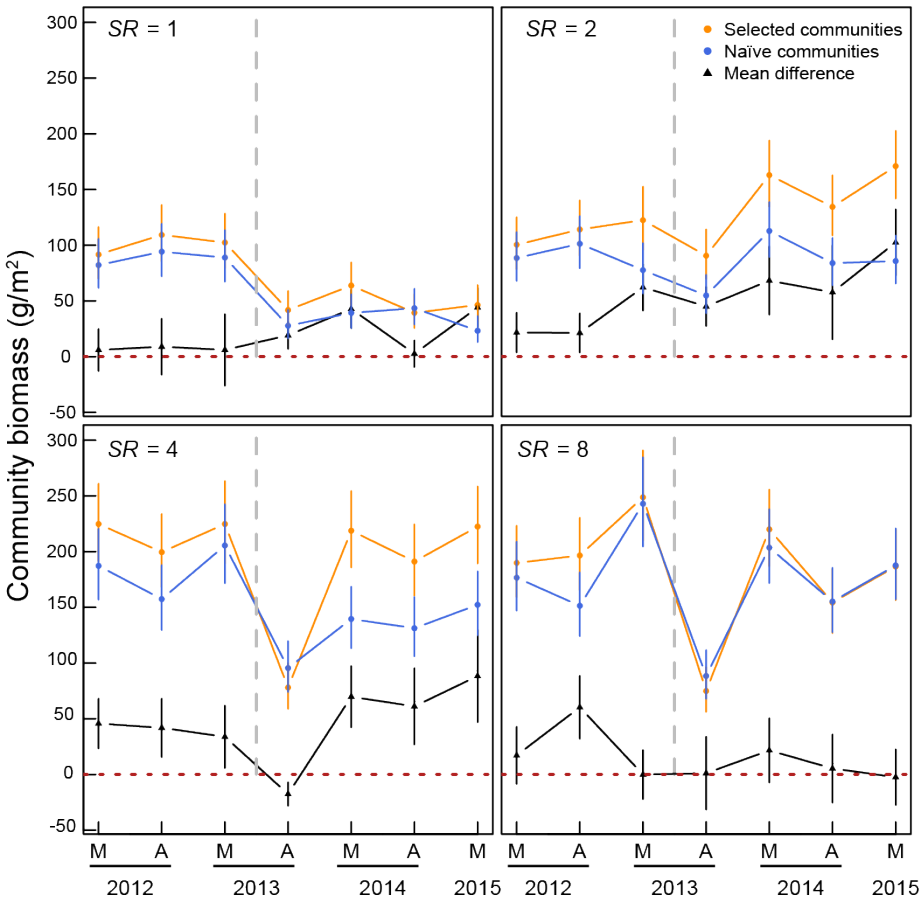

Fig. 1. Aboveground community biomass over time at four species richness levels (SR). Selected and naïve plant communities and their mean difference are plotted with means and standard errors calculated from raw data. The dashed line indicates the flooding event. M = May, A= August.

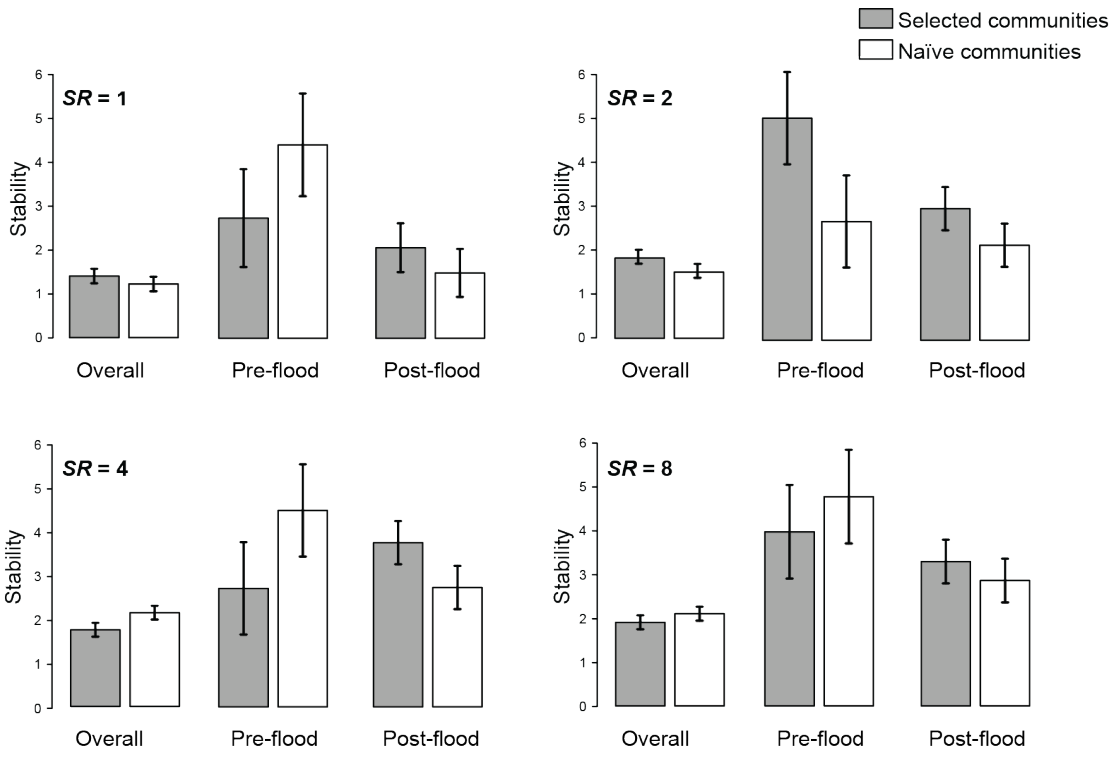

Fig. 2. Temporal stability of aboveground community biomass calculated as mean divided by standard deviation over all seven harvests, and for three pre-flood and three post-flood harvest dates for selected (dark grey) and naïve (white) plant communities at four species richness levels (SR). Means and standard errors calculated from raw data are shown.

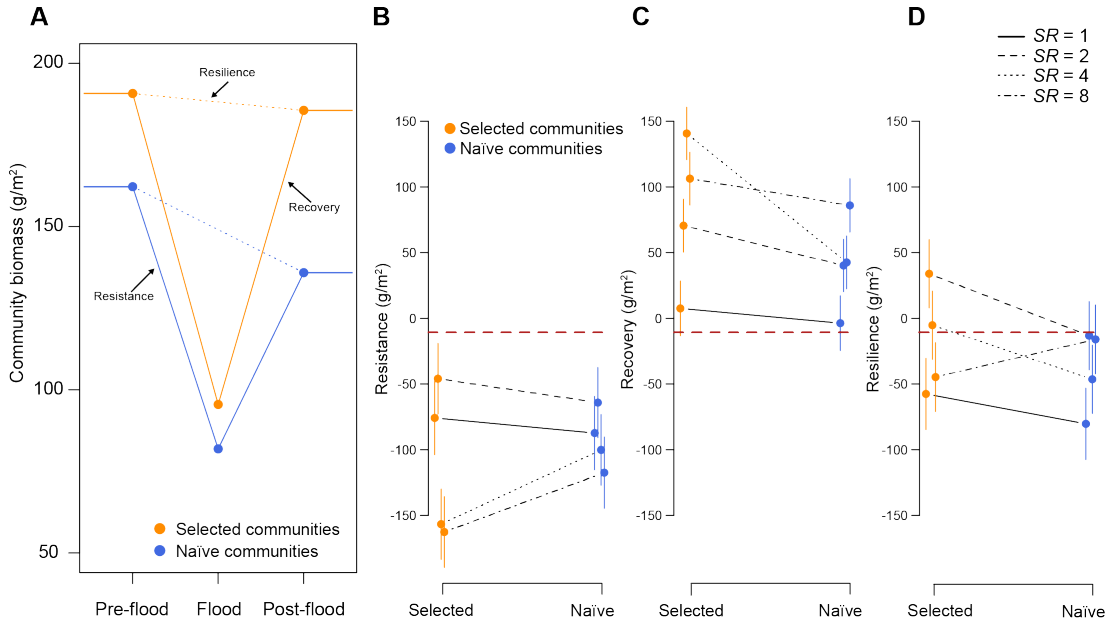

Fig. 3. (*A*) Aboveground community biomass of the three pre-flood harvests, the harvest directly after the flooding event and the three post-flood harvests and the three derived measures resistance, recovery and resilience. (*B*) Absolute resistance of selected vs. naïve plant communities ($F = 2.697$, $P = 0.103$ for main effect of plant history) at four species richness levels ($F = 5.992$, $P = 0.016$ for interaction of plant history with log species richness). (*C*) Absolute recovery of selected vs. naïve plant communities ($F = 15.2$, $P < 0.001$ for main effect of plant history) in four species richness levels ($F = 0.987$, $P = 0.322$ for interaction of plant history with log species richness). (*D*) Absolute resilience of selected vs. naïve plant communities ($F = 2.485$, $P = 0.117$ for main effect of plant history) in four species richness levels ($F = 1.869$, $P = 0.174$ for interaction of plant history with species richness). Model estimated means and standard errors are shown.

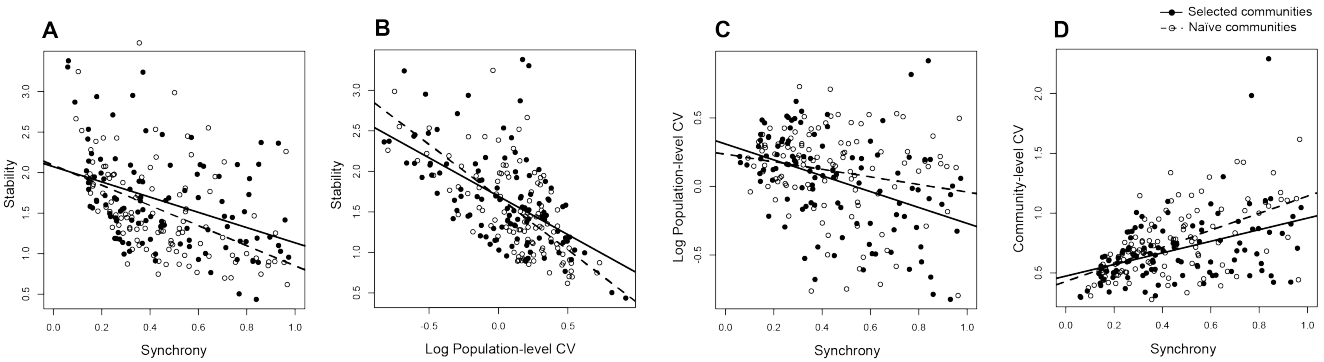

Fig. 4. Population-level mechanisms underlying the observed stability of aboveground community biomass for selected (solid line, black circles) and naïve (dashed line, open circles) communities. (*A*) Community stability as a function of population-level synchrony ($F = 89.25$ and $P < 0.001$ for the regression and $F = 1.23$, $P = 0.270$ for the interaction with the community-evolution treatment). (*B*) Community stability as a function of log-transformed population-level variance ($F = 187.7$ and $P < 0.001$ for the regression and $F = 3.94$, $P = 0.049$ for the interaction with the community-evolution treatment). (*C*) Log-transformed population-level variance as a function of population-level synchrony ($F = 1.099$ and $P = 0.296$ for the regression and $F = 5.229$, $P = 0.023$ for the interaction with the community-evolution treatment). (*D*) Log-transformed community-level coefficient of variance (inverse of community stability) as a function of population-level synchrony ($F = 105.9$ and $P < 0.001$ for the regression and $F = 3.628$, $P = 0.059$ for the interaction with the community-evolution treatment). Monocultures were excluded from all the population-level analyses.

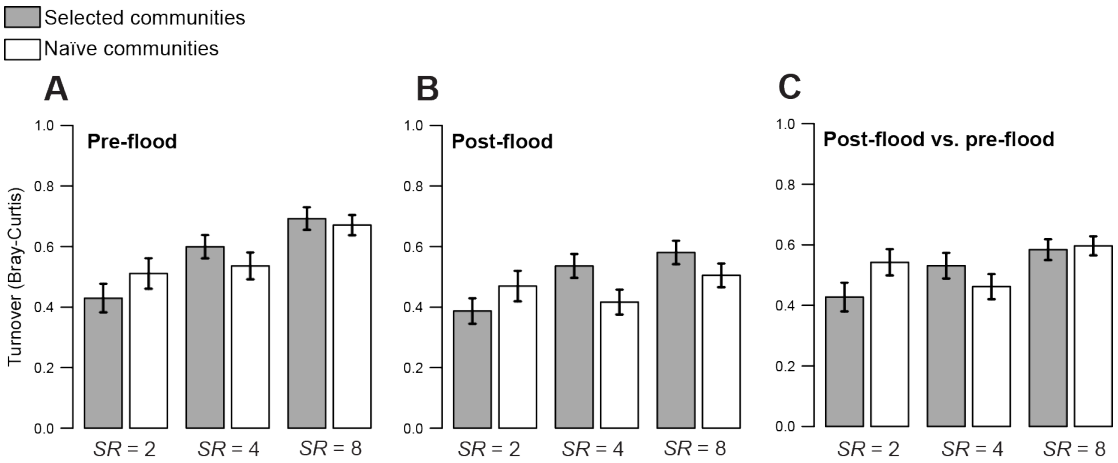

Fig. 5 Compositional turnover (Bray-Curtis dissimilarity) for selected (dark grey) and naïve (white) communities at three species richness levels (SR). (*A*) Turnover during the three pre-flood harvests, (*B*) turnover during the three post-flood harvests, and (*C*) turnover post-flood versus the turnover pre-flood. Means and standard errors calculated for raw data are shown.

Table 1. Results of linear mixed-effects ANOVA for pre-flood, post-flood and overall stability (see text) of the test communities in terms of aboveground biomass. Stability measures were log-transformed to achieve variance homogeneity. Significant effects ($P < 0.05$) are highlighted in bold font.

Pre-flood stability	Df	denDF	*F*	*P*
Log species richness (logSR)	1	44.5	5.775	**0.020**
Soil treatment (ST)	2	88	0.169	0.845
Plant history (PH)	1	135.9	1.89	0.171
SH × logSR	2	89.6	0.941	0.394
PH × logSR	1	137.2	1.084	0.300
Post-flood stability				
Log species richness (logSR)	1	43.9	13.48	**< 0.001**
Soil treatment (ST)	2	86.3	1.025	0.363
Plant history (PH)	1	133.6	4.359	**0.039**
ST × logSR	2	87.1	0.236	0.791
PH × logSR	1	134.1	0.094	0.759
Overall stability				
Log species richness (logSR)	1	45.2	14.66	**< 0.001**
Soil treatment (ST)	2	89.2	0.237	0.790
Plant history (PH)	1	138	0.002	0.969
ST × logSR	2	89.3	0.285	0.753
PH × logSR	1	138	7.929	**0.006**

Note: nDF = numerator degrees of freedom, dDF = denominator degrees of freedom, F = variance ratio, P = probability of type-I error.

Table 2. Result of linear mixed-effects ANOVA for the absolute resistance, recovery and resilience of the test communities. Significant effects ($P < 0.05$) are highlighted in bold font.

Resistance	nDf	dDF	*F*	*P*
Log species richness (logSR)	1	44.9	5.563	**0.023**
Soil treatment (ST)	2	87.2	14.92	**< 0.001**
Plant history (PH)	1	136.5	2.697	0.103
ST × logSR	2	86.9	6.241	**0.003**
PH × logSR	1	136.1	5.992	**0.016**
Recovery				
Log species richness (logSR)	1	42.2	17.18	**< 0.001**
Soil treatment (ST)	2	88.1	0.305	0.738
Plant history (PH)	1	135.6	15.2	**< 0.001**
ST × logSR	2	87.9	1.522	0.224
PH × logSR	1	135.2	0.987	0.322
Resilience				
Log species richness (logSR)	1	43	0.3422	0.562
Soil treatment (ST)	2	88.7	8.51	**< 0.001**
Plant history (PH)	1	138	2.485	0.117
ST× logSR	2	88.9	6.905	**0.002**
PH × logSR	1	138	1.869	0.174

Note: nDF = numerator degrees of freedom, dDF = denominator degrees of freedom, F = variance ratio, P = probability of type-I error.

Table 3. Result of linear mixed-effects ANOVA for stability of aboveground community biomass, log-transformed population-level variance and log-transformed community-level variance (inverse of community stability). Significant effects ($P < 0.05$) are highlighted in bold font.

Stability	nDf	dDF	*F*	*P*
Synchrony	1	200.5	89.25	**< 0.001**
Plant history (PH)	1	104.1	5.33	**0.023**
Synchrony × PH	1	130.5	1.23	0.270
Stability				
Log Pop-CV	1	195.8	187.7	**< 0.001**
Plant history (PH)	1	106.1	0.32	0.575
Log Pop-CV × PH	1	131.4	3.94	**0.049**
Log Population-CV				
Synchrony	1	206.6	1.099	0.296
Plant history (PH)	1	173.5	4.028	**0.046**
Synchrony × PH	1	176.7	5.229	**0.023**
Log Community-CV				
Synchrony	1	203.5	105.9	**< 0.001**
Plant history (PH)	1	103.9	6.399	**0.013**
Synchrony × PH	1	134.6	3.628	0.059

Note: nDF = numerator degrees of freedom, dDF = denominator degrees of freedom, F = variance ratio, P = probability of type-I error. Monocultures were excluded for these analyses.

Supporting Information

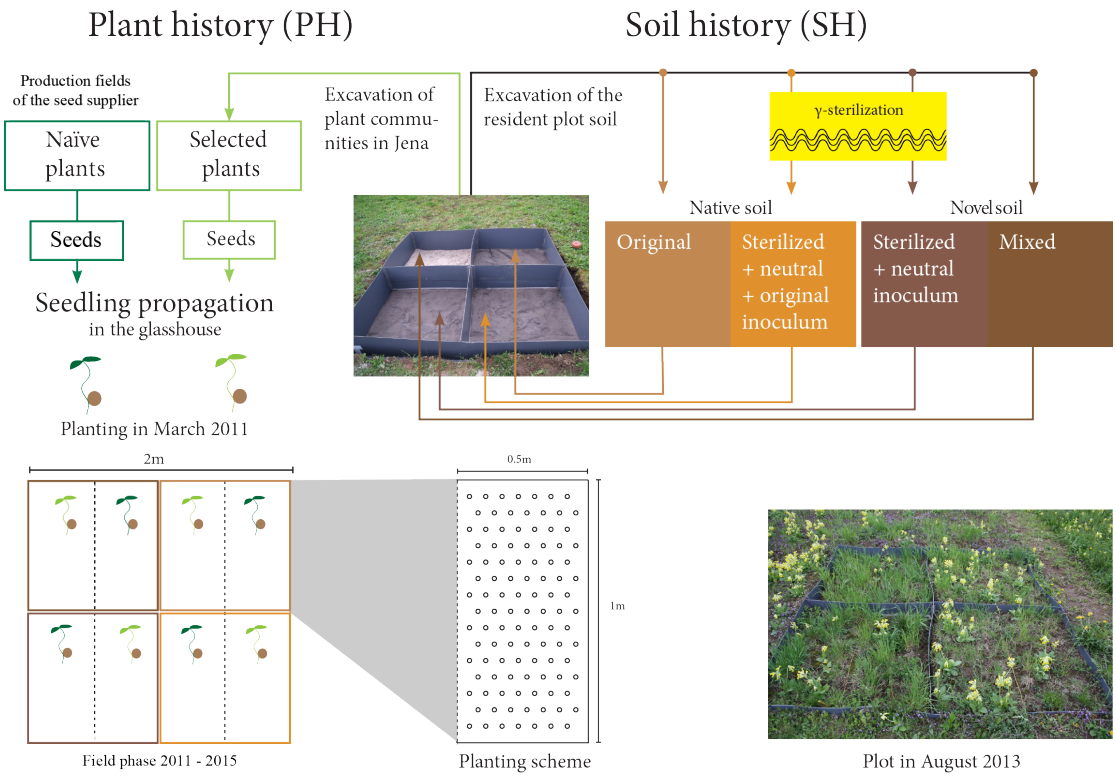

Fig. S1. Experimental design. In a glasshouse, plants from two community-evolution treatments were propagated. Selected plants were propagated from seeds of plants, which were previously excavated from their communities in the experimental field; naïve plants were propagated from seeds purchased from a seed supplier. Subsequently, the seedlings were planted in the field according to randomized planting schemes with equal species densities. Communities of selected plants (light green) and of naïve plants (dark green) were grown in four different soil treatments filled into quadrats (shades of brown), either sterilized or unsterilized, and either containing native soil (with co-selected soil biota) or not. One of the four soil treatments (mixed soil) was forgone after two years of the experiment because the plants were used for a different experiment. Data from this fourth treatment were therefore excluded from all analyses presented in this paper.

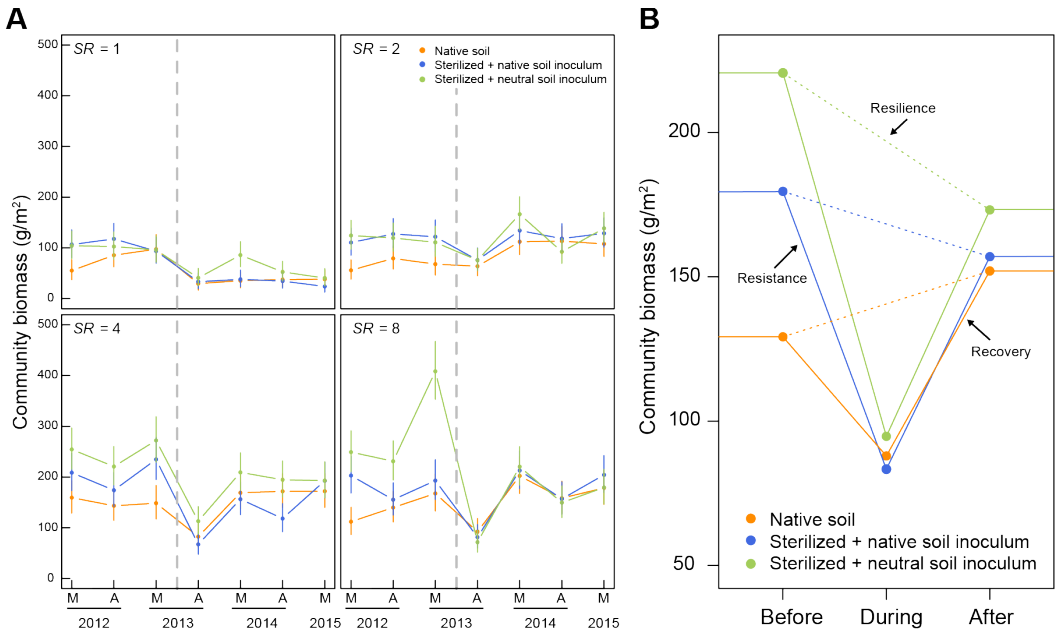

Fig. S2. Influence of the soil treatment on stability of aboveground community biomass. (*A*) Aboveground community biomass over time at four species richness levels (SR) in three soil treatments. Means and standard errors were calculated for raw data. The dashed line indicates the flooding event. M = May, A= August. (*B*) Aboveground community biomass of the three pre-flood harvests ("Before"), the harvest directly after the flooding event ("During") and the three post-flood harvests ("After") and the three derived measures resistance, recovery and resilience in three soil treatments. Model estimated means and standard errors are shown.

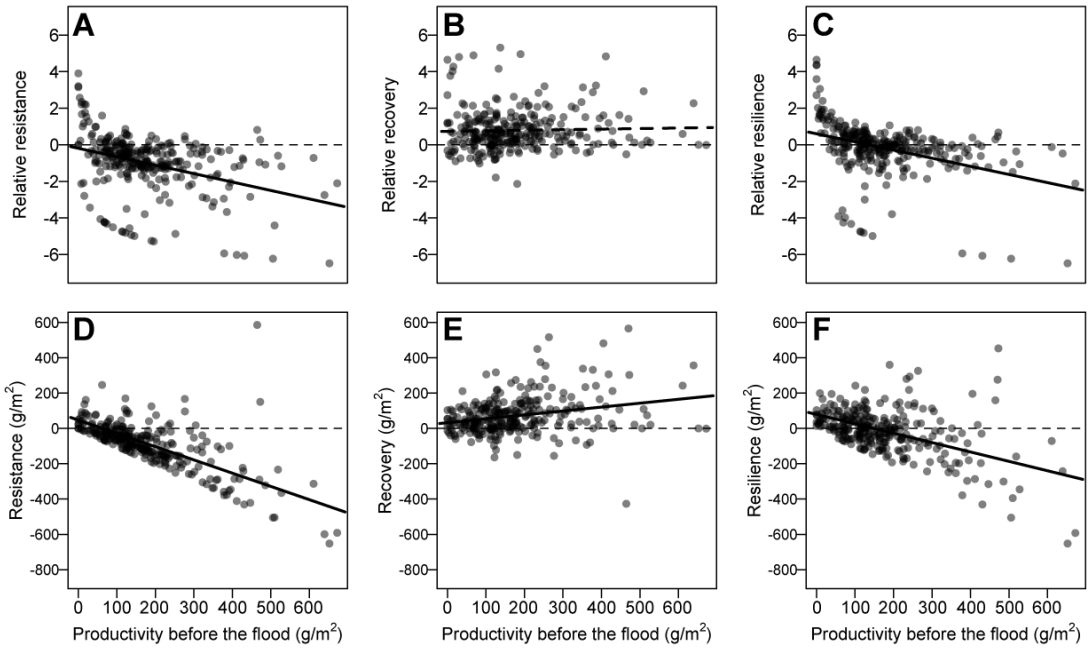

Fig. S3. Influence of mean pre-flood aboveground community biomass on resistance, recovery and resilience. (*A*) Relative resistance ($F_{1,198.9}$ = 32.90, P < 0.001). (*B*) Relative recovery ($F_{1,240.6}$ = 0.108, P = 0.743). (*C*) Relative resilience ($F_{1,196.7}$ = 29.540, P < 0.001. (*D*) Absolute resistance ($F_{1,195.2}$ = 266.200, P < 0.001) (*E*) Absolute recovery ($F_{1,218.1}$ = 10.550, P = 0.001). (*F*) Absolute resilience ($F_{1,228.6}$ = 122.90, P < 0.001). Non-significant relationship indicated by a dashed line.

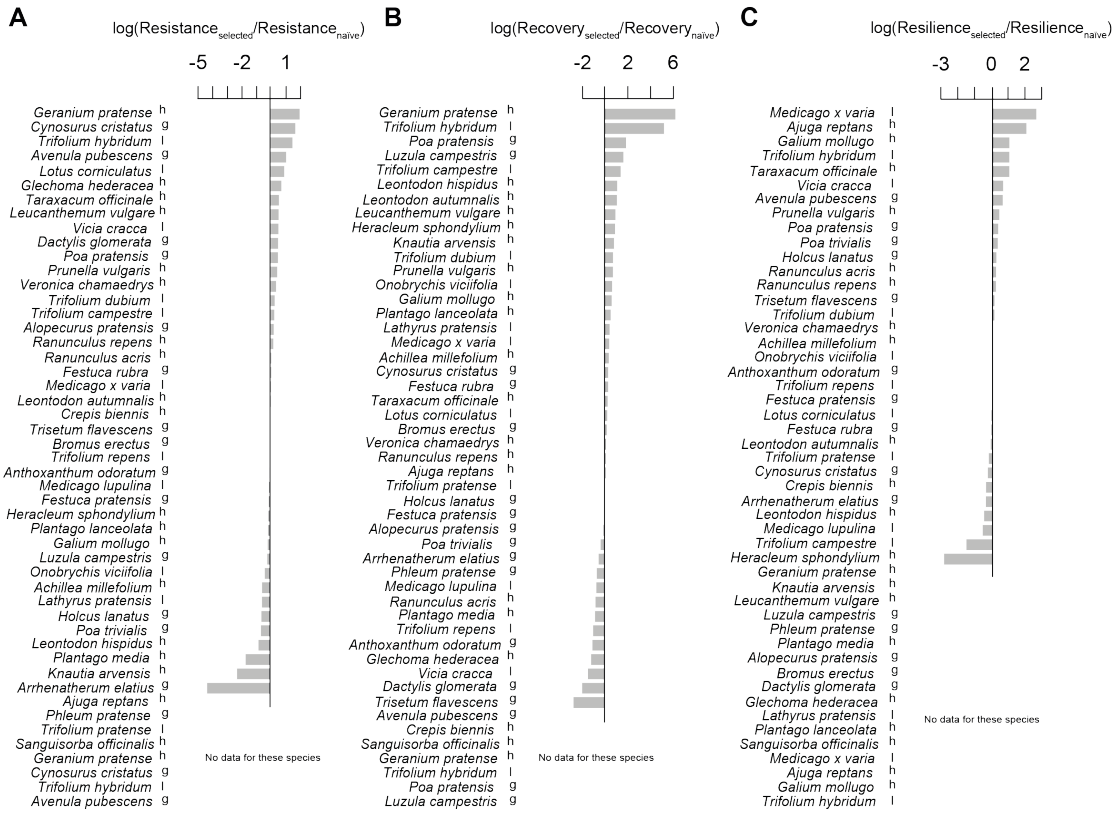

Fig. S4. Log-ratio between resistance, recovery and resilience of selected and naïve test communities for each species separately. (*A*) Log-ratio of absolute resistance, (*B*) log-ratio of absolute recovery and (*C*) log-ratio of absolute resilience. l: legume, h: herb, g: grass.

Table S1. Result of linear mixed-effects ANOVA for the relative resistance, recovery and resilience of the test communities. Significant effects ($P < 0.05$) are highlighted in bold font.

Relative resistance	nDf	dDF	*F*	*P*
Log species richness (logSR)	1	45.1	0.0034	0.954
Soil treatment (ST)	2	84	4.861	**0.010**
Plant history (PH)	1	132.6	1.279	0.260
ST × logSR	2	83.7	2.802	0.066
PH × logSR	1	132.1	5.338	**0.022**
Relative recovery				
Log species richness (logSR)	1	44.9	3.383	0.072
Soil treatment (ST)	2	85	0.232	0.794
Plant history (PH)	1	132	4.522	**0.035**
ST × logSR	2	84.8	0.166	0.848
PH × logSR	1	131.6	5.397	**0.022**
Relative resilience				
Log species richness (logSR)	1	45.1	1.412	0.241
Soil treatment (ST)	2	89.1	4.961	**0.009**
Plant history (PH)	1	138	0.6622	0.417
ST × logSR	2	89.1	3.444	**0.036**
PH × logSR	1	138	0.118	0.732

Note: nDF = numerator degrees of freedom, dDF = denominator degrees of freedom, F = variance ratio, P = probability of type-I error.

Table S2. Result of linear mixed-effects ANOVA for mean species turnover, pre-flood turnover, post-flood turnover, and pre-flood vs. post-flood turnover. Turnover calculated using the Bray-Curtis dissimilarity. Significant effects ($P < 0.05$) are highlighted in bold font.

Mean (pre- and post-flood)	nDf	dDF	*F*	*P*
Log species richness (logSR)	1	34.2	6.29	**0.017**
Plant history (PH)	1	102.9	0.98	0.325
PH × logSR	1	102.9	5.74	**0.018**
Turnover pre-flood	nDf	dDF	*F*	*P*
Log species richness (logSR)	1	33.9	6.749	**0.014**
Plant history (PH)	1	104.2	0.021	0.886
PH × logSR	1	104	2.504	0.117
Turnover post-flood	nDf	dDF	*F*	*P*
Log species richness (logSR)	1	33.9	2.92	0.097
Plant history (PH)	1	102	1.71	0.194
PH × logSR	1	102	5.18	**0.025**
Turnover pre- vs. post-flood	nDf	dDF	*F*	*P*
Log species richness (logSR)	1	34	2.234	0.144
Plant history (PH)	1	105	0.62	0.433
PH × logSR	1	105	2.886	0.092

Note: nDF = numerator degrees of freedom, dDF = denominator degrees of freedom, F = variance ratio, P = probability of type-I error. Monocultures were excluded from this analysis.

CHAPTER THREE

Selection in response to community diversity alters plant performance in newly assembled test communities

Selection in response to community diversity alters plant performance in newly assembled test communities

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Currently under review at *Ecology*

Author contributions

B.S., S.J.V.M. and D.Z.D. conceptualized the project; S.J.V.M. and T.H. carried out the experiment; M.W.S., S.J.V.M. and B.S. analysed the data; S.J.V.M. wrote the first draft of the manuscript. All authors contributed to the final manuscript.

Abstract. In grassland biodiversity experiments the positive biodiversity–ecosystem functioning relationship generally increases over time. However, there is still a large gap in our understanding of the underlying short-term evolutionary processes. Research has shown that differential selection in monoculture vs. mixed-species communities can lead to rapid evolution. We assessed whether selection history altered productivity, biodiversity effects and species complementarity within newly assembled monocultures and 2-species mixtures using five plant species selected for twelve years in such a biodiversity experiment in mixture or monoculture and plants without such a selection history. Plants without past community selection history produced the lowest community biomass and showed the weakest biodiversity effects. Furthermore, we found that twelve years of selection history in monocultures or species mixtures differentiated plants into monoculture- and mixture-types within species. In newly assembled mixtures, plants with a selection history in mixtures performed better than plants with a monoculture selection history. Biodiversity effects were generally positive but, contrary to expectation, not stronger for mixture types. In addition, biodiversity effects were both influenced by trait differences among plants and community-weighted means, but these relationships were largely independent of selection history. Our findings indicate possible mechanisms underlying the rapid evolution of adapted subtypes within a species in grasslands. Uncovering these mechanisms contributes to our understanding of the biodiversity–ecosystem functioning relationship, which has the potential to influence species conservation practice.

Key words biodiversity effects, complementarity effect, ecosystem functioning, grasslands, plant productivity, sampling effect, species selection, trait variation

INTRODUCTION

The loss of biodiversity due to species extinctions is a major threat to global ecosystems (Steffen et al. 2015) and has led to a large body of research investigating the importance of biodiversity to maintain ecosystem functions, such as productivity or nutrient cycling (Cardinale et al. 2012). In grasslands, many studies have found a positive biodiversity–productivity relationship (e.g. Tilman et al. 2001, Isbell et al. 2011), with biodiversity increasing multiple ecosystem functions (Soliveres et al. 2016) and ecosystem services (Balvanera et al. 2006). The positive effect of biodiversity has also been shown to increase with time (Cardinale et al. 2007, Reich et al. 2012), suggesting that complementarity between the co-occurring species can increase over time (Fargione et al. 2007).

Despite more than a decade of research on the biodiversity–productivity relationship (e.g. Reich et al. 2012), little is known about evolutionary mechanisms potentially affecting species interactions (Thorpe et al. 2011). It is conceivable that selection acting on traits could increase ecological combining ability (Harper 1977, Aarssen 1983) via niche differentiation in plant mixtures (Zuppinger-Dingley et al. 2014). Such adaptation may occur when there is either sufficient standing genetic variation in a population and the most suitable genotypes are sorted out (Fakheran et al. 2010) or by recombination and novel mutations (Anderson et al. 2011). Furthermore, plants may adapt to a novel environment by phenotypic plasticity (Price et al. 2003, Turcotte and Levine 2016), thus changing their morphology without genotypic changes. Epigenetic mechanisms have been suggested to enable adaptation (Bossdorf et al. 2008), especially in short-term evolutionary processes.

Whereas the influence of environmental factors on adaptive responses of plant populations is well studied (e.g. Schmid 1985, Joshi et al. 2001), much less effort has been devoted to studying the influence of community diversity on a species' performance (but see Lipowsky et al. 2011, Kleynhans et al. 2016). Based on previous observations in experimental ecosystems suggesting a "division of labor" among species in plant mixtures, it is likely that community diversity plays a role in the evolution of plant functional trait variation. For example, in forests more diverse tree communities have been shown to express greater crown complementarity (Niklaus et al. 2017, Williams et al. 2017). In diverse grassland communities, increased complementarity effects as estimated by the additive partitioning method of Loreau and Hector (2001) have been observed promoting community productivity via a range of mechanisms: diversification of the canopy structure and hence light and space use (Spehn et al. 2000, Allan et al. 2011), soil resource partitioning (Fornara and Tilman 2008, Roscher et al. 2008, von Felten et al. 2009), root depth distribution (Mueller et al. 2013) and distribution of leaf mass (Wacker et al. 2009). It is now timely to ask how and on what time scale selective forces may contribute to the evolution of the observed combining ability.

Using the additive partitioning method (Loreau and Hector 2001), net biodiversity effects (NEs) can be partitioned into complementarity (CEs) and sampling effects (SEs). When CEs drive over-yielding, most species are expected to

contribute to greater biomass in more diverse communities. In contrast, when SEs are drive over-yielding, a few dominant species increase community productivity in species mixtures. The CE is therefore related to coexistence and trait variation between species, as it inherently suggests a differentiation in functional traits (Cadotte et al. 2009, Flynn et al. 2011). Conversely, the SE should rather be driven by traits of the dominant species and thus by community-weighted trait means (CWMs); an increase in CWMs (e.g. taller plants) should increase biodiversity effects (Roscher et al. 2012).

The use of functional traits to define species' niches has a long history in evolutionary ecology (van Valen 1965, Schoener and Gorman 1968, Roughgarden 1974) but only recently has become a popular approach in functional ecology (Violle et al. 2007) where it is being used to explain mechanisms of species coexistence and ecosystem functioning (Kraft et al. 2015, Hart et al. 2016). However, there is still a large gap in our understanding of how evolutionary mechanisms shape such trait-based niches (Roscher et al. 2015) and how they may drive corresponding niche differentiation according to functional traits (Sterck et al. 2011).

In particular, the selective power of community diversity on biodiversity effects as well as trait means and variation has received limited attention (but see Lipowsky et al. 2011, Zuppinger-Dingley et al. 2014, 2015, 2016, Kleynhans et al. 2016, Rottstock et al. 2017). Kleynhans et al. (2016) observed adaptation to new environmental conditions but only when the diversity level of the selection treatment and the assay treatment were the same. Although a selective past of growth in different diversity levels has been shown to have trans-generational influences on productivity for one species (Rottstock et al. 2017), it is unknown whether such an effect may be common to many species in plant communities.

In the present study, we tested whether community diversity as a selective environment can influence heritable variation in plant aboveground biomass and functional traits within and between species and how this may relate to biodiversity effects in two-species mixtures. We measured biomass production and traits of individual plants in monocultures and mixtures established with seedlings from either a selection history of experimental monoculture or mixture communities in a biodiversity field experiment (Jena Experiment, see (Roscher et al. (2004) for methods) or in monoculture fields from the commercial seed supplier which provided the original seeds for the biodiversity experiment in 2002. We refer to the plants growing in Jena since 2002 in mixture or monoculture experimental plots as mixture types and monoculture types, respectively. The plants derived from seeds obtained from the commercial supplier in 2014 are referred to as naïve plants.

Whereas selection outcomes in an earlier study in the Jena Experiment were assessed after eight years and one controlled sexual reproduction cycle (Zuppinger-Dingley et al. 2014), in the present study we continued the selection treatment for another four years and added a second controlled sexual reproduction cycle. We included naïve plants as a control treatment without selection under continuous growth in monoculture or mixture communities. We hypothesized that during the

twelve years of selection in the experimental field, mixture-type plants should have evolved high mixture performance (hypothesis 1, see Table 1). This should be related to large NEs, in particular CEs (hypothesis 2), and large between-species trait variation (hypothesis 3). Conversely, we hypothesized that monoculture-type plants should have evolved high monoculture performance (hypothesis 4), which should be related to large within-species trait variation (hypothesis 5). For control plants, we hypothesized intermediate results between monoculture- and mixture-type plants. We therefore aimed to expand on the relationship between biodiversity effects and between-species trait variation, hypothesizing that large CEs should be due to between-species trait variation (hypothesis 6). Finally, we hypothesized that large SEs should be due to large CWMs (hypothesis 7).

METHODS

Plant selection histories

To test whether plant types selected over twelve years in mixtures outperform those types selected in monocultures when assembled in mixture test communities, we chose five species grown in monoculture and mixture plots in the Jena Experiment (Jena, Thuringia, Germany, 51˚N, 11˚E, 135 m a.s.l., see Roscher et al. (2004) for experimental details): *Plantago lanceolata* L.*, Prunella vulgaris* L.*, Veronica chamaedrys* L., *Galium mollugo* L. and *Lathyrus pratensis* L. For brevity, we will use the genus names to refer to the species. The study species had previously been classified into the following functional groups (Roscher et al. 2004): *Veronica*, *Prunella* and *Plantago* as small herbs, *Galium* as a tall herb and *Lathyrus* as a legume.

Plant progeny from three different selection histories was used for the experiment. Plants without selection history in the Jena Experiment (selection history "naïve") were obtained from a commercial seed supplier (Rieger Hoffmann GmbH, Blaufelden-Raboldshausen, Germany). Plants with a selection history in the Jena Experiment were grown in either mixtures or monocultures from 2002 (selection history "mixture" and "monoculture", respectively). In 2010, cuttings of these plants were brought to Zurich and used for seed production for an earlier experiment. The plants were grown in their respective community in an experimental garden in Zurich and seeds were collected from these plants throughout the growing season of 2010. The propagation of seedlings from these seeds is described in Zuppinger-Dingley et al. (2014). These seedlings were then planted back into the experimental plots in Jena in 2011 using the identical parental species composition (for detailed procedure see van Moorsel et al. (2017)).

To ensure a second sexual reproductive event and to collect seed material for the present study, entire plant communities from some of the experimental plots replanted in Jena in 2011 were excavated again in March 2014. These plants were used to establish plots with an identical plant composition to the plots in Jena from which the plants were collected, in an experimental garden in slug-exclosure compartments at the University of Zurich, Switzerland (47°33′N, °37′E, 534 m a.s.l.).

We added a layer of soil ("Gartenhumus" consisting of 50% agricultural soil and 50% garden compost, Ricoter, Aarberg, Switzerland) to each plot to ensure the plants established well. Mesh fabric netting around each plot minimized the possibility of cross-pollination between the same species from different experimental plots. Seeds were collected throughout the growing season of 2014 from monoculture plots and from 4- and 8-species mixtures. Seeds from different mother plants were pooled together. Seeds were cleaned manually for three species and mechanically for two species (*Plantago* and *Prunella*). The dry seeds were stored at 5° C for cold stratification until germination.

Experimental set up

Seeds were germinated in germination soil ("Anzuchterde", Ökohum, Herbertingen, Germany) under constant conditions in the glasshouse without additional light in December 2014, each species being sown on the same day. From 25 February to 13 March 2015, seedlings were planted in monocultures of four individuals and 2-species mixtures of four individuals into pots (two liters) filled with neutral agricultural soil (50% agricultural sugar beet soil, 25% perlite, 25% sand; Ricoter AG, Aarberg, Switzerland). Seedlings which died in the first two weeks were replaced with seedlings of the same species and age.

These seedlings were used to assemble test communities in six blocks (replicates) with each block consisting of the full experimental design as far as possible. Within each block, pots were placed on three different tables in the glasshouse in a randomized fashion without reference to selection history or assembly treatment. Throughout the experiment, we did not move pots but noted their position in the glasshouse. Single pots always contained four plants of the same selection history. Every selection history × species assembly combination was replicated five to six times depending on plant availability. We planted 30 monoculture- and 42 mixture-assemblies with mixture selection history, 30 monoculture and 60 mixture assemblies with monoculture selection history and 24 monoculture and 35 mixture assemblies with naïve selection history. There were thus 221 pots and 884 plants (Appendix S2 for monoculture identities and species combinations).

During the experiment, we grew the plants initially at day temperatures of 17–20°C and night temperatures of 13–17°C without supplemental light. To compensate for overheating in summer, an adiabatic cooling system (Airwatech; Bern, Switzerland) was used to match inside with outside temperatures. The plants were not fertilized. Due to an infestation of white flies (*Trialeurodes vaporariorum*, Westwood 1856) and spider mites (*Tetranychidae* spp., Donnadieu 1875), we applied the insecticide SanoPlant Neem (1% Azadirachtin A (10 g/l); Maag AG) three times. The fungicide Fenicur (*Oleum foeniculi*, Andermatt Biocontrol) against powdery mildew (*Podosphaera* spp.) was applied twice. Plant height, leaf thickness, specific leaf area (SLA) and individual aboveground biomass were measured after twelve weeks of the experiment from 18 May to 4 June 2015. Leaf thickness was measured for three representative leaves using a thickness gauge. Specific leaf area (SLA) of up to 20

representative leaves (depending on the leaf size of the species) of each species in a pot was measured by scanning fresh leaves with a Li-3100 Area Meter (Li-cor Inc., Lincoln, Nebraska, USA) immediately after harvest and determining the mass of the same leaves after drying. Plant height and individual aboveground biomass were measured a second time after 24 weeks, the end of the experiment, from 18–25 August 2015. All four individuals in a pot were sampled. Research assistants, who were not informed of the specific experimental treatments, assisted in the regular measurements and harvesting of plants at the end of the experiment.

Data analyses

SLA outliers (> 99% percentile) were replaced with a maximum value (the 99% percentile, n = 6). We calculated pot-wise aboveground community biomass (plant community production) as the sum of the biomass of the four individual plants. Relative between-species differences (RDs, absolute difference between two species divided by the mean of the two) in plant height (first and second harvest), leaf thickness (first harvest) and SLA (first harvest) were calculated for mixture assemblies. Relative differences within species were calculated for both mixture and monoculture assemblies taking the relative difference between two individuals of the same species per pot. Furthermore, we calculated community-weighted means (CWMs) and pot standard deviation (SDs) for the same traits. Pots with dead plant individuals were excluded from the calculation of community-weighted means, but were included for the other data analyses. Net biodiversity effects (NEs) were calculated by comparing the 2-species mixtures with the average monoculture and partitioned according to Loreau and Hector (2001) into complementary (CEs) and sampling (selection) effects (SEs). This partitioning approach allows assessing how CEs and SEs contribute to the observed NEs (Loreau and Hector 2001). To avoid confusion with the term selection used for the selection-history treatment, we here use the term "sampling effect" for the SE (as in Zuppinger-Dingley et al. (2014)). Additive partitioning calculations were based on the difference between the observed yield of each species in the mixture and the monoculture yield for that species and selection history averaged across blocks. Absolute values of CE and SE were square root-transformed and the original signs put back on the transformed values for analysis (Loreau and Hector 2001). Differences in these measures between mixtures assembled from plants with monoculture selection history and mixtures assembled from plants with mixture selection history would suggest differential evolution of trait-based niches between species as a potential mechanism underlying biodiversity effects.

All statistical analyses were done in R (Version 3.2.3, R Core team 2016). Mixed-model analysis was done using the R-package asreml (VSNI international, 2016) and results assembled in ANOVA tables. Fixed-effects terms were selection-history treatment (naïve, monoculture, mixture), assembly treatment (monoculture vs. 2-species mixture assemblies), species identity of monoculture assemblies and of mixture assemblies (in short "species assembly") and interactions of these. Table

(including blocks) was used as random-effects term. CWMs, RDs, within species differences and SDs of plant height, SLA and leaf thickness were added as covariates to models to investigate the influence of these covariates on community biomass and biodiversity effects.

RESULTS

Plant selection history and community productivity

We compared the community productivity of plants from different selection histories (naïve, monoculture, mixture) grown in newly assembled monocultures and 2-species mixtures by harvesting aboveground biomass twice, once after twelve weeks and a second time after 24 weeks. Because the first measure assessed growth and the second regrowth, the harvests were analyzed separately. Communities consisting of plants with naïve selection history produced the lowest community biomass at both the first and the second harvest (Fig. 1, Table 2). At the second harvest, this contrast between plants with and without selection history was stronger in mixture than in monoculture assemblies (Fig 1; interaction monoculture vs. mixture × naïve vs. monoculture or mixture in Table 2). Hence, both plants with monoculture- (unexpected) and with mixture- (expected) selection history in the Jena Experiment benefitted more from growing in mixtures (see also analysis of biodiversity effects in the next section).

At the second harvest the mixture-selection-history communities outperformed the monoculture-selection-history communities and this effect was marginally more pronounced in mixture assemblies (see Fig.1 and main effect mono types vs. mix types and the two-way interaction monoculture vs. mixture assembly × mono vs. mix types in Table 2). This partly confirms hypothesis 1 but not hypothesis 4 (see hypotheses listed in Table 1).

Species identity in monoculture or mixture assemblies strongly influenced community productivity and, especially at the first harvest, the interaction terms with selection history were significant (main effect monoculture identity or species assembly of mixture and two-way interactions species assembly × naïve vs. mono or mix types and species assembly × mono types vs. mix types in Table 2). For example, at the first harvest, mixture-type plants performed better than monoculture-type plants in newly assembled monocultures of *Prunella* (rejecting hypothesis 4) and in mixtures of *Galium* and *Prunella* (confirming hypothesis 1) (Fig. 1a). However, in the two mixtures with the small herbs *Veronica* and *Prunella* and *Plantago* and *Prunella*, monoculture-type plants performed better than mixture-type plants (rejecting hypothesis 1; see Fig. 1a).

Plant selection history and biodiversity effects

Overall, biodiversity effects were positive at both harvests (First harvest: NE: $F_{1,15.9} = 26.67$, $P < 0.001$, CE: $F_{1,15.8} = 8.214$, $P = 0.011$, SE: $F_{1,14.2} = 97.07$. $P < 0.001$, second harvest: NE: $F_{1,15.1} = 14.35$, $P = 0.002$, CE: $F_{1,14.5} = 4.108$, $P = 0.061$,

SE: $F_{1,15.1}$ = 11.66, P = 0.004, Fig. 2, Appendix S3 and S4). At the first harvest, communities of naïve plants on average showed larger SEs than communities of selected plants ($F_{1,104.2}$ = 12.66, P = 0.001, Appendix S3). At the second harvest, however, NEs and CEs were significantly lower for naïve plant communities (NE: $F_{1,96.1}$ = 11.54, P < 0.001, CE: $F_{1,96.6}$ = 5.668, P = 0.019, Appendix S4). These results are in line with the results obtained for community productivity: plant communities consisting of plants without selection history had the lowest average productivity mainly because they could profit the least from growing in 2-species mixtures rather than in monocultures. We had expected naïve plants to have intermediate biodiversity effects between monoculture- and mixture-type plants.

Contrary to our expectation (hypothesis 2), at the first harvest NEs, CEs and SEs were significantly larger for communities assembled from monoculture-type plants than for communities assembled from mixture-type plants (NE: $F_{1,93.9}$ = 21.01, P < 0.001; CE: $F_{1,94.4}$ = 14.2, P < 0.001; SE: $F_{1,101.2}$ = 10.28, P = 0.002; Appendix S3; Fig. 2a–c, upper panels). This difference was reversed for most species assemblages at the second harvest (Fig. 2a–c, lower panels), when NE, CE and SE were non-significantly larger for communities assembled from mixture-type plants (Appendix S4). In line with the results obtained for community productivity, the influence of selection history on biodiversity effects also additionally depended on the specific species combination in mixture assemblies as follows (interactions species assembly × naïve vs. mono or mix types and species assembly × mono types vs. mix types in Appendix S3 and S4). At the first harvest, we found the expected result (hypothesis 2), i.e. a larger NE for mixtures types, for the combinations of *Galium* with either *Prunella* or *Plantago* (Fig. 2a, upper panel). At the second harvest, NEs and CEs were generally more similar between selection histories across different combinations and variation between the specific community compositions was mainly due to different SEs. An exception was the combination *Galium* + *Prunella*, which similarly to the first harvest showed a much larger NE for mixture-type plants, as expected under hypothesis 2. When both harvests were considered, communities including the legume *Lathyrus* or the small herb *Plantago* showed positive biodiversity effects (Fig. 2; effects of species assembly in Appendix S3 and S4). When comparing the CEs between the first and the second harvest, we found that four species combinations shifted from stronger biodiversity effects for monoculture types (rejecting hypothesis 2) to stronger biodiversity effects for mixture types (supporting hypothesis 2) (Fig. 2b). The *Galium* + *Prunella* species combination showed a consistently larger CE for mixture-type plants (supporting hypothesis 3). At the second harvest the different species combinations varied strongly in SEs, but not in CEs (CE: $F_{9,98.4}$ = 1.121, P = 0.356, SE: $F_{9,100.8}$ = 11.53, P < 0.001, Appendix S4). SEs were often larger for mixture than for monoculture types (Fig. 2c).

Plant selection history and within- and between-species trait variance

For SLA, plant height (at the first and at the second harvest) and leaf thickness we calculated relative differences within and between species as well as the total pot

standard deviation (SD) and tested for differences between two of the three selection-history treatments (contrast between mixture- and monoculture-type plants, Fig. 3). The difference in plant height at the first harvest was marginally greater interspecifically for plants selected in mixtures, in accordance with hypothesis 3. In contrast to this hypothesis, the interspecific relative difference in leaf thickness was greater for plants selected in monocultures. Monoculture types showed greater intraspecific relative difference in SLA, in accordance with hypothesis 5. Furthermore, pot-level SDs in monocultures (were it was expected under hypothesis 5) or mixture assemblies were non-significantly larger for communities assembled with monoculture than with mixture types (see left two columns in Fig. 3).

Relationship between biodiversity effects and plant functional traits

We tested how the biodiversity effects were related to the measured functional trait variation (hypothesis 6; Fig. 4 and Fig. 5) and their means (hypothesis 7; Fig. 6 and Fig. 7). We calculated community-weighted means (CWMs) for plant height, SLA and leaf thickness at the first harvest and for plant height at the second harvest. We then related these CWMs to the partitioned biodiversity effects and analyzed if and how selection history could influence this relationship.

First, we looked at the relationship of biodiversity effects with between-species differences (RDs) for SLA, plant height and leaf thickness in mixture pots (Fig. 4). The NE was negatively correlated with the RD of plant height and positively correlated with the RD of leaf thickness (see Fig. 4). Thus, while biodiversity effects decreased with increasing variation in plant height, they increased with increasing variation in leaf thickness. Contrary to our expectations under hypothesis 6, this was mainly driven by the SE, whereas the CE was less influenced by the RDs. Selection history had an effect on the relationship between biodiversity effects and RDs marginally or significantly. SEs were more negatively correlated with the RDs of plant height for mixture- than the RDs of monoculture-type and naïve plants. In contrast, the RD of leaf thickness was positively correlated with NEs and CEs for both monoculture and mixture types, but not for naïve plants (Fig. 4c). At the second harvest, NEs and SEs were significantly negatively correlated with the RD of plant height (Fig. 5). CEs were not influenced by interspecific variation in plant height, again not supporting hypothesis 6. SLA and leaf thickness were not measured at the second harvest.

Next, we looked at the relationship of biodiversity effects with community-weighted trait means CWMs). Whereas CEs were negatively correlated with the CWM of SLA (Fig. 6b), the SE was positively correlated with SLA (Fig. 6b, right panel). Consequently, NEs, driven by CEs, decreased with increasing SLA. Leaf thickness had a marginally significant effect on SEs, but the directionality depended on selection history. Plant height did not have a significant effect on any of the biodiversity effects at the first harvest. However, the interaction between trait means

and selection history was significant for the relationship between the CWM of plant height and the SE at the first harvest. Thus, even though the trait mean did not have a direct impact on biodiversity effects, selection history influenced the trait means, which in turn influenced biodiversity effects. Selection history did not significantly impact the relationship between biodiversity effects and CWMs for the other two traits. At the second harvest, CWM of plant height had a significantly positive effect on NE, CE and SE (Fig. 7), hence the biodiversity effects were stronger for overall taller plants. However, when compared to the first harvest, the effect of selection history on the relationship between the CWM of plant height and the SE disappeared at the second harvest (Fig. 7). Overall, these results provided mixed evidence for hypothesis 7, which predicted a positive relationship between SEs and CWMs but no relationship between CEs and CWMs.

DISCUSSION

Influence of plant selection history on community productivity (hypotheses 1 and 4)

Previous research has shown that plant community productivity can be influenced by plant selection history, especially by the selection for increased niche differentiation in plants that had been grown for eight years in mixtures (mixture-type plants) compared to plants that had been grown in monoculture (monoculture-type plants, Zuppinger-Dingley et al. 2014). The present study included naïve plants without selection history in a biodiversity experiment. For plants with a selection history in the Jena biodiversity experiment (Roscher et al. 2004), we hypothesized that 2-species mixtures newly assembled with mixture-type plants should have greater community productivity than similar mixtures newly assembled with monoculture-type plants (hypothesis 1) and, conversely, that monocultures newly assembled with monoculture-type plants should have greater community productivity than similar monocultures newly assembled with mixture-type plants (hypothesis 4). For naïve plants, we expected intermediate community productivity in both monocultures and mixtures.

Our results provide mixed evidence for these hypotheses, in part depending on the particular species and species combinations. Thus, plant communities consisting of naïve plants without a selection history in the Jena Experiment often produced the lowest community biomass, especially in 2-species mixtures (see Fig. 1). It is conceivable that evolutionary processes in the field plots, where plants were grown for a longer time without re-sowing than was the case for the naïve plants in the propagation cultures of the commercial supplier, led to the increased performance of selected plants.

Comparing test communities consisting of either monoculture-type plants or mixture-type plants, we observed that mixture-type plants did have higher community productivity than monoculture-type plants in 2-species mixtures, as expected under hypothesis 1. But contrary to our expectation (hypothesis 4), mixture-type plants also

produced more biomass than monoculture-type plants when grown in monoculture, thereby reducing biodiversity effects as discussed below. The generally lower performance of monoculture-type plants could have been due to selection for increased defense, trading off with reduced growth (Coley et al. 1985, Herms and Mattson 1992). The increased defense may not have become effective during the 24 weeks of growth in the present experiment. In a parallel glasshouse experiment with single individuals per pot, we indeed found greater pathogen damage on mixture- than on monoculture-type plants (Terhi Hahl et al., personal observation).

Within these main effects of selection history, we found large variation in selection-history effects among species in monocultures and among species compositions in 2-species mixtures. These findings emphasized the importance of conducting such studies with multiple species but at the same time sufficient replication for each in monoculture and for their combinations in mixture. High replication can more easily be achieved in experiments with one focal species (e.g. Kleynhans et al. 2016, Rottstock et al. 2017), but extrapolating results from such experiments might under- or overestimate overall effects of selection on the response of plants to different biotic conditions. In the present study, we used five focal species and already found strong differences regarding their selection response to community diversity.

Influence of plant selection history on biodiversity effects (hypothesis 2)

Net biodiversity effects (NEs) can be partitioned into CEs and SEs. When CEs drive over-yielding, most species should contribute equally contribute to greater community productivity in mixtures, due to niche differentiation among them. Conversely, SEs are large when few dominant species are driving positive diversity–productivity relationships, because they benefit from growing in mixtures (Loreau and Hector 2001).

Naïve plants exhibited weak biodiversity effects, confirming findings from a field experiment (van Moorsel et al. 2017), where we found biodiversity effects to be weaker for communities assembled with naïve plants, especially when comparing monocultures with 2- and 4-species mixtures. As mentioned above, naïve plants in contrast to selected plants had not experienced the continued selection in field plots without re-sowing. Furthermore, they had not experienced interspecific competition before, which was at least the case for the monoculture types among the selected plants. Comparing the monoculture and mixture types, we found that at the first harvest NEs, CEs and SEs were larger for communities consisting of monoculture-type plants, which for the NEs and CEs was in contrast with our expectation (hypothesis 2). The lower CE for mixture-type plants was due to the good performance of mixture types in newly assembled monocultures and not because mixture types performed poorly in newly assembled mixtures. At the second harvest, NEs, CEs and SEs were rather similar for the two selection histories, thus no longer contradicting expectations, but also not supporting them (hypothesis 2). Nevertheless, at least in four 2-species combinations — *Lathyrus* + *Veronica*, *Galium* + *Veronica*,

Veronica + *Prunella* and *Plantago* + *Prunella* — the directionality changed from the unexpected to the expected result, i.e. CEs at the second harvest were larger for mixture- than monoculture-type plants (see Fig. 2b). Over longer timespans, CEs often increase and SEs often decrease (van Ruijven and Berendse 2005, Fargione et al. 2007, Montès et al. 2008, Isbell et al. 2009, Marquard et al. 2009). It is conceivable that this would also have occurred in our experiment if it had continued beyond the 24-weeks timespan.

Influence of plant selection history on trait variation (hypotheses 3 and 5)

Because community-level trait variation can reflect niche differentiation (Violle et al. 2012, Roscher et al. 2015), we measured intra- and interspecific trait variation among individual plants in all communities. We hypothesized that mixture-type plants should exhibit larger trait variation between species as they underwent selection for increased complementarity during twelve years in the experimental field plots (hypothesis 3). Conversely, we expected stronger within-species trait variation in monoculture-type plants, due to 12 years of strong intraspecific competition in the experimental field plots (hypothesis 5). Overall, we found that variation tended to be larger both within and between species for monoculture-type plants (see Fig. 3), thus not confirming hypothesis 3, but weakly confirming hypothesis 5. Several studies have investigated the relationship between species richness and community-level trait variation (Hulshof et al. 2013, Le Bagousse-Pinguet et al. 2014, Lamanna et al. 2014, Siefert et al. 2015) and found that the relative extent of intraspecific trait variation depended on species richness. In monocultures, a large intraspecific variation is advantageous for a more efficient use of resources, leading to our hypothesis 5. Thus, the observed trend for increased trait variation in monoculture types (see Fig. 3) is consistent with potential selection for within-species niche differentiation and character displacement in monocultures.

The lack of increased between species trait differences in mixture- compared with monoculture-type plants was in accordance with a lack of increased CEs for mixture-type plants. This contrasts with the results of an earlier study in which increased CEs of mixture-type plants were associated with increased between-species trait differences (Zuppinger et al. 2014). A potential explanation for the different results is that the earlier study used species which were more different among each other, namely grasses, legumes, small herbs and tall herbs, whereas species in the present study were more similar and therefore perhaps less likely to further increase their differences by short-term evolution than species which were more different to begin with. The species in the present study may have evolved “parallel” character displacement in response to species of the other functional groups also present in the mixtures in which they were selected in the Jena Experiment.

Influence of trait variation and community-weighted means on biodiversity effects (hypotheses 6 and 7)

One potential underlying mechanism for increased biodiversity effects observed in field experiments (Cardinale et al. 2007, Reich et al. 2012), could be selection for niche differentiation (Zuppinger-Dingley et al. 2014). Not all trait variation, however, corresponds to niche differentiation (Turcotte and Levine 2016). In particular, traits related to light availability may behave differently because of the asymmetric nature of competition for light, i.e. being tall is generally better than being small. Thus, variation in plant height could be expected to decrease when species are grown in mixtures rather than monocultures (Vermeulen et al. 2008, Roscher et al. 2015). Given the absence of increased CEs and between-species trait variation in mixture-type plants, the relationship between functional traits in our 2-species mixtures and biodiversity effects should not have differed according to plant selection history. Nevertheless, we could still test how trait variation and means were correlated with biodiversity effects. Specifically, we predicted that relative trait differences (RDs) should be positively related to CEs (hypothesis 6) and community-weighted trait means (CWMs) should be positively related to SEs (hypothesis 7).

In opposition to hypothesis 6, RDs in plant height were negatively rather than positively correlated with CEs and consequently NEs (see Fig. 4a, 5). This discrepancy of observation and expectation suggests that RDs in plant height may reflect competitive hierarchies rather than complementary of plants with respect to light use, as discussed above with regard to the asymmetry of light competition. At the second harvest, CWMs of plant height had a positive impact on all biodiversity effects (Fig. 7), i.e. not only on SEs — which we had expected under hypothesis 7 —, but in accordance with findings of previous studies (Vermeulen et al. 2008, Roscher et al. 2015).

Functional diversity in SLA within a community should increase complementary light use (Roscher et al. 2011). Leaf thickness is inherently related to SLA (White and Montes-R 2005) and might act similarly to SLA. In our study, RDs in leaf thickness, but not RDs in SLA, were positively correlated with all biodiversity effects, especially for mixture-type plants (see Fig. 4c). Hence, trait plasticity in leaf thickness was advantageous for species growing in mixtures. However, SEs was as much increased as CEs, whereas according to our expectation (hypothesis 6) positive correlations between trait differences should mainly involve CEs. Additionally, CWMs of SLA did have a positive effect on SEs, consistent with hypotheses 7, but also a negative effect on CEs, adding up to a negative effect on NEs (see Fig. 6b), suggesting that overall a smaller leaf area per unit mass for species growing in mixtures has a positive effect on productivity.

CONCLUSIONS

Here, we demonstrated that community diversity had the selective potential to alter species performances, which may in part explain the strengthening biodiversity–ecosystem functioning relationship observed in the field experiments (e.g. Reich et al. (2012)). Selection in a biodiversity experiment increased community productivity in newly assembled test communities compared to communities consisting of naïve plants without such selection history. Moreover, selection in mixtures increased community productivity in newly assembled mixtures and monocultures compared with selection in monocultures. These findings imply that co-evolutionary processes occurred throughout the 12-year selection period in the experimental plots of the biodiversity experiment and involving at least two sexual reproduction cycles.

Selection experiments like the present one should include a number of species and species compositions, because these may show different evolutionary responses, as observed in the present study. Studies with one focal species might either under- or overestimate the effects of "biodiversity selection" on the response to current assay conditions. Revealing such rapid evolutionary processes in grassland plant communities also has implications for conservation strategies. Thus, it may not be sufficient to only conserve species in isolation but rather in communities or populations of species with co-evolved interactions.

ACKNOWLEDGEMENTS

We thank T. Zwimpfer, M. Furler, D. Trujillo and D. Topalovic for technical assistance and E. de Luca, N. Castro and M. Brezzi for help with data measurements. This study was supported by the Swiss National Science Foundation (grants number 147092 and 166457 to B. Schmid) and the University Research Priority Program Global Change and Biodiversity of the University of Zurich. The Jena Experiment is supported by the German Science Foundation (FOR 1451, SCHM 1628/5-2). S.J. van Moorsel, B. Schmid and D. Zuppinger-Dingley conceived the study, S.J. van Moorsel and T. Hahl carried out the experiment and S.J. van Moorsel, M.W. Schmid and B. Schmid analyzed the data. S.J. van Moorsel and B. Schmid wrote the manuscript with all other authors contributing to revisions.

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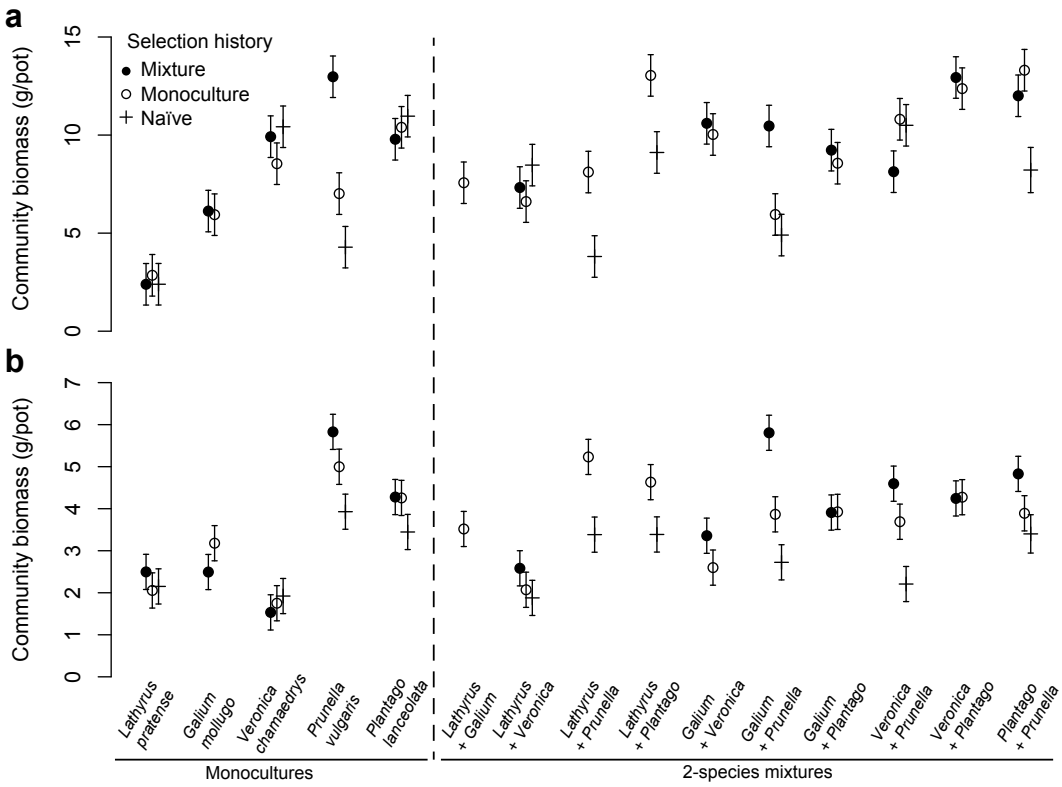
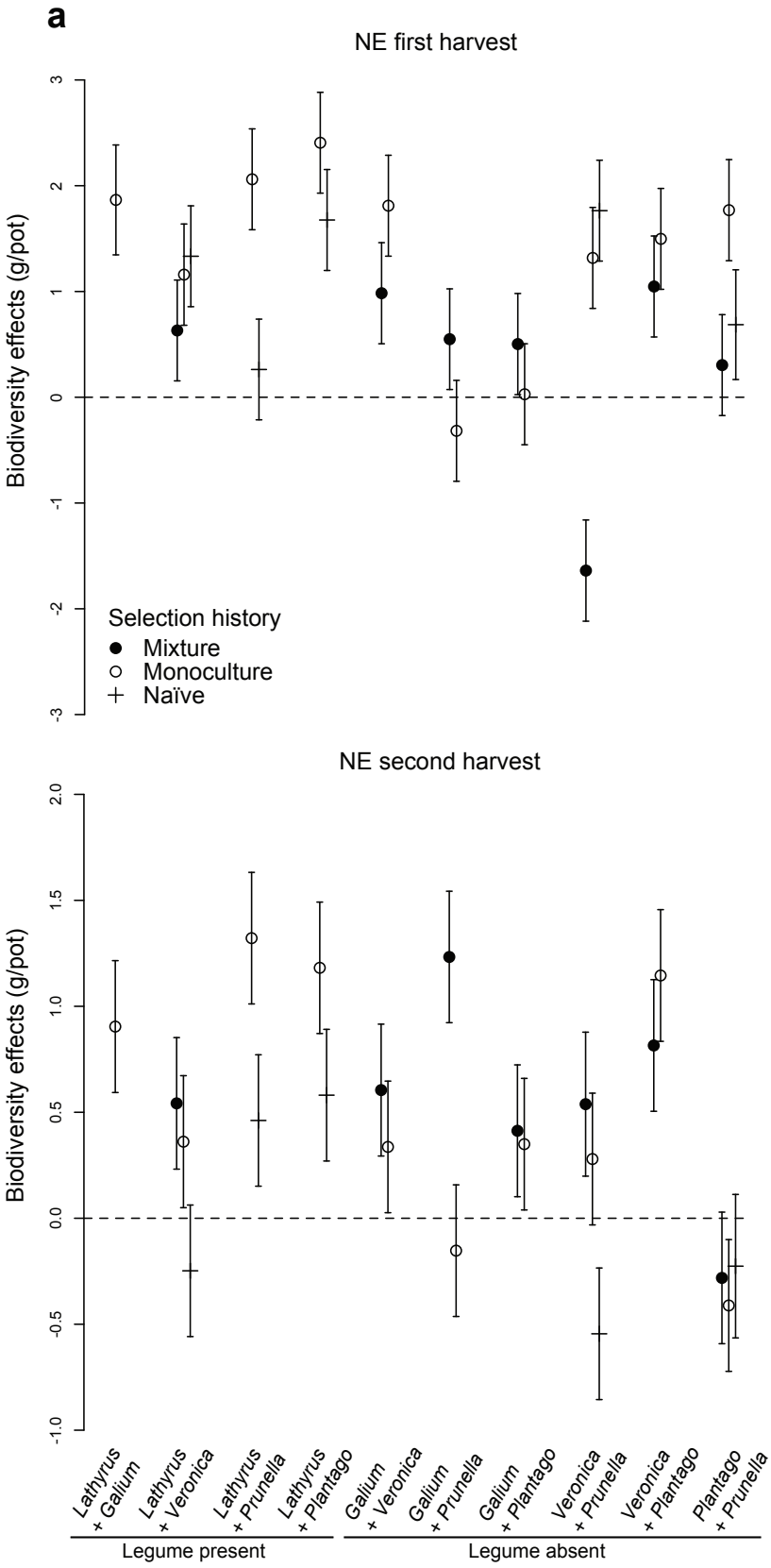
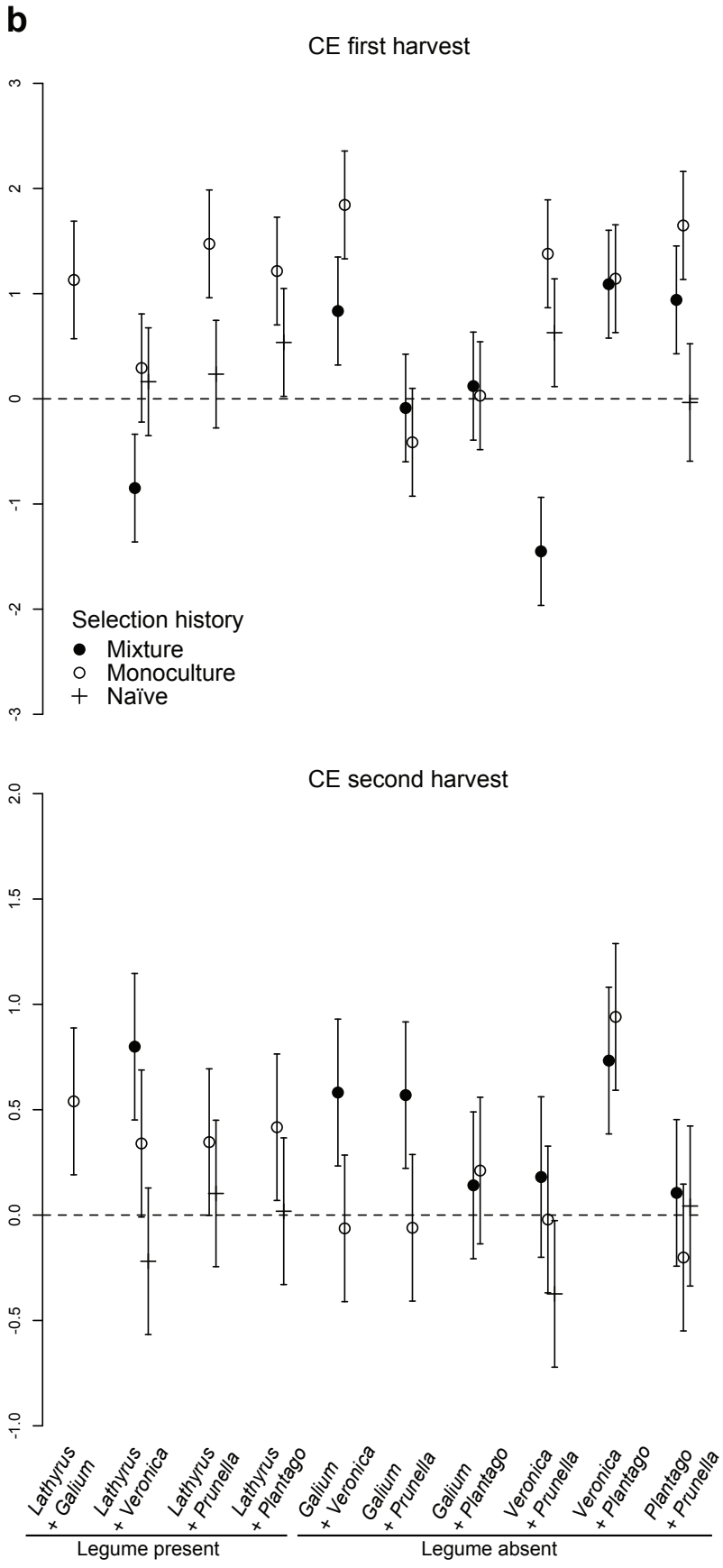


FIG. 1. Mean community biomass for monocultures and 2-species mixtures. Shown are means and standard errors from a linear mixed-effects model with selection history, species combination and the interaction between selection history and species assembly as fixed-effects terms and table (including the block) as random-effects term. **a**, first harvest. **b**, second harvest.


a
NE first harvest
Biodiversity effects (g/pot)
3
2
1
0
-1
-2
-3
Selection history
Mixture
Monoculture
Naïve
NE second harvest
Biodiversity effects (g/pot)
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
Lathyrus + Galium
Lathyrus + Veronica
Lathyrus + Prunella
Lathyrus + Plantago
Galium + Veronica
Galium + Prunella
Galium + Plantago
Veronica + Prunella
Veronica + Plantago
Plantago + Prunella
Legume present
Legume absent


b
CE first harvest
3
2
1
0
-1
-2
-3
Selection history
Mixture
Monoculture
Naïve
CE second harvest
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
Lathyrus + Galium
Lathyrus + Veronica
Lathyrus + Prunella
Lathyrus + Plantago
Galium + Veronica
Galium + Prunella
Galium + Plantago
Veronica + Prunella
Veronica + Plantago
Plantago + Prunella
Legume present
Legume absent

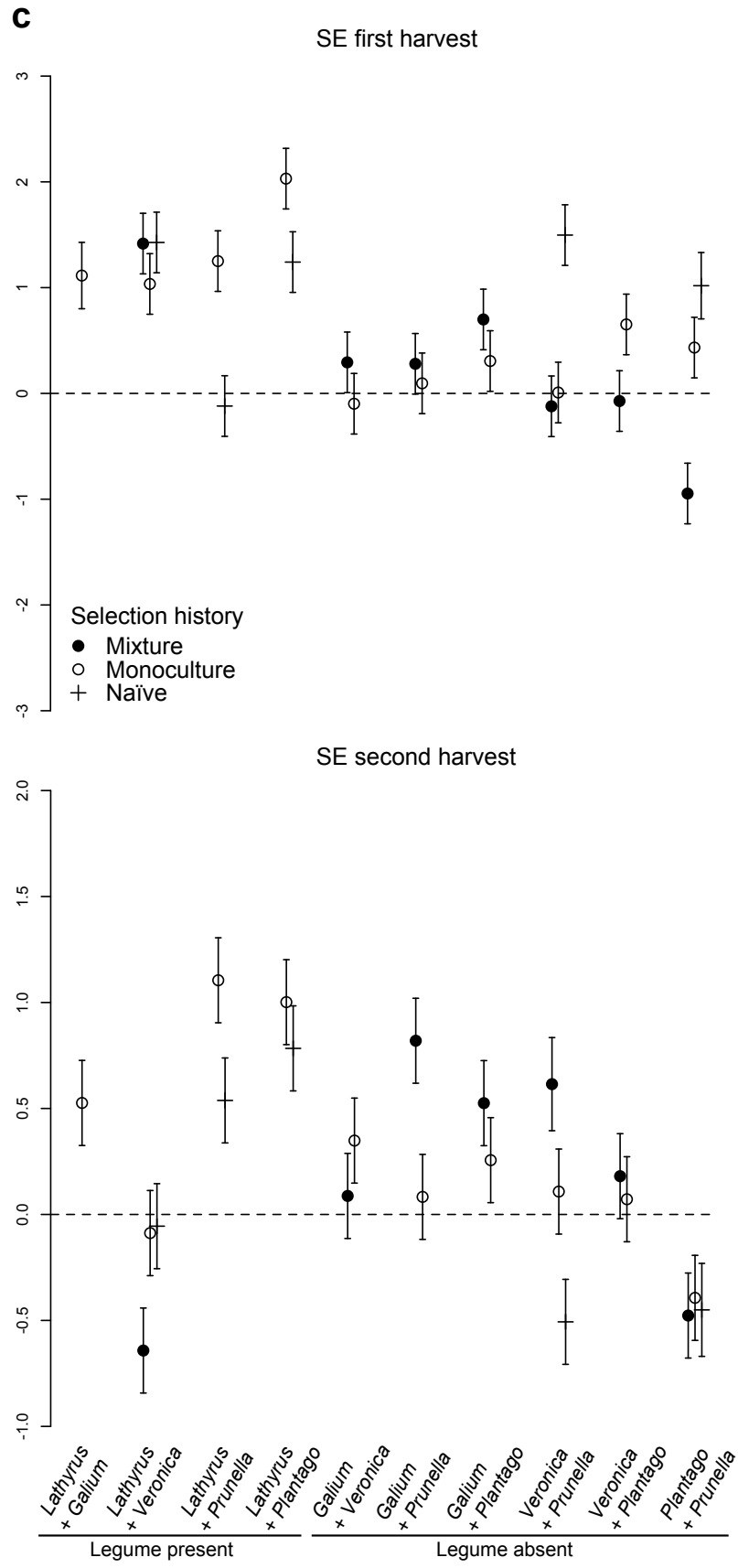

c
SE first harvest
3
2
1
0
-1
-2
-3
Selection history
Mixture
Monoculture
Naïve
SE second harvest
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
Lathyrus + Galium
Lathyrus + Veronica
Lathyrus + Prunella
Lathyrus + Plantago
Galium + Veronica
Galium + Prunella
Galium + Plantago
Veronica + Prunella
Veronica + Plantago
Plantago + Prunella
Legume present
Legume absent

FIG. 2. Biodiversity effects were assessed for both biomass harvests by additive partitioning of the net effect (**a,** NE) into complementarity effect (**b,** CE) and sampling effect (**c,** SE) for plants with different selection histories (naïve, monoculture, mixture). Shown are means and standard errors from a linear mixed-effects model, with selection history, species assembly and the interaction between selection history and species assembly as fixed-effects terms and table (including block) as random-effects term.

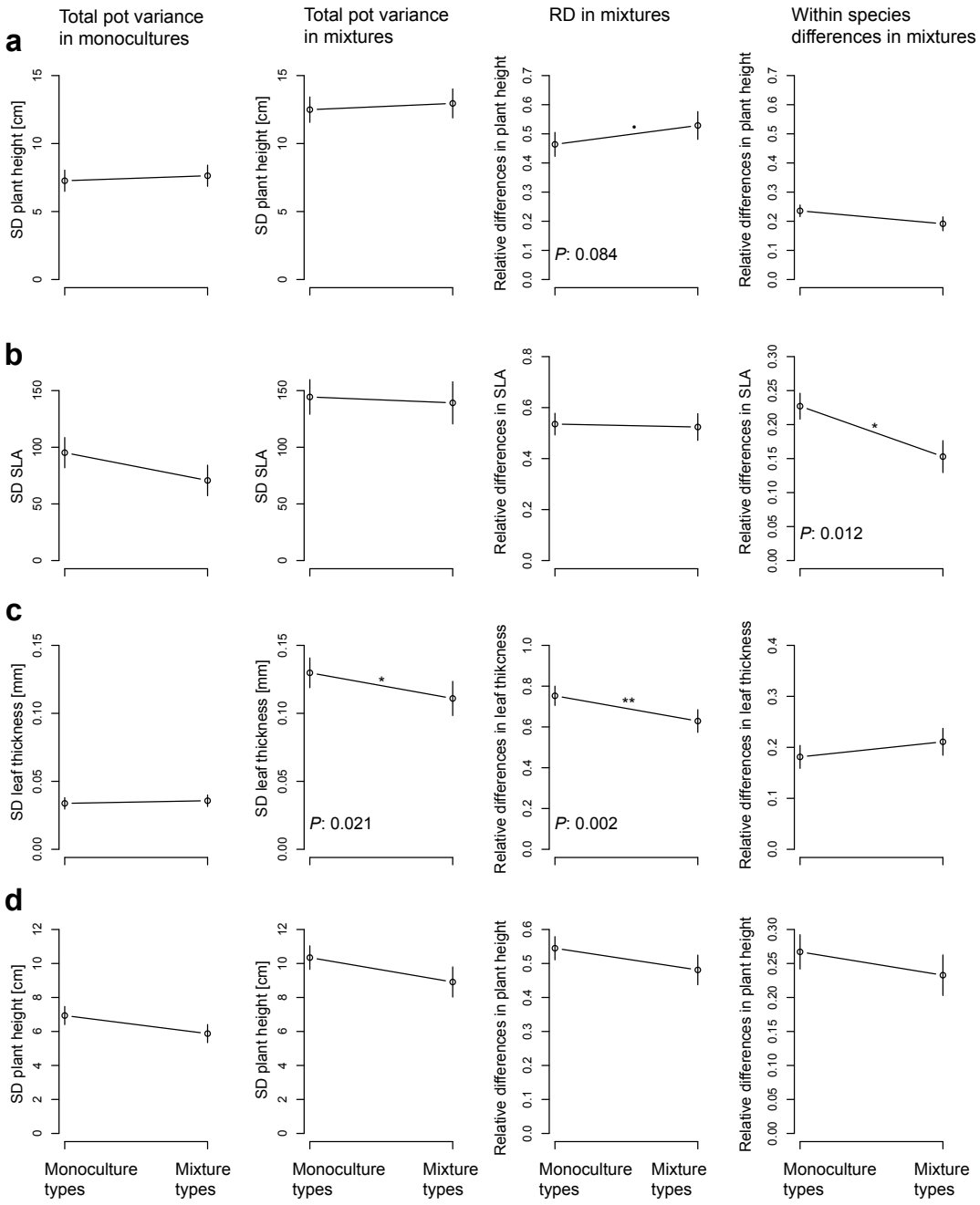


FIG. 3. Trait variance in monoculture and mixture assemblies in response to selection history (monoculture- vs. mixture-type plants). **a)** plant height at the first harvest, **b)** SLA at the first harvest, **c)** leaf thickness at the first harvest, **d)** plant height at the second harvest. Shown are means and standard errors from a mixed-effects model with selection history, species assembly and the two-way interaction of these as fixed-effects terms and table (including block) as random term. Significant and marginally significant *P*-values are indicated in the respective plot.

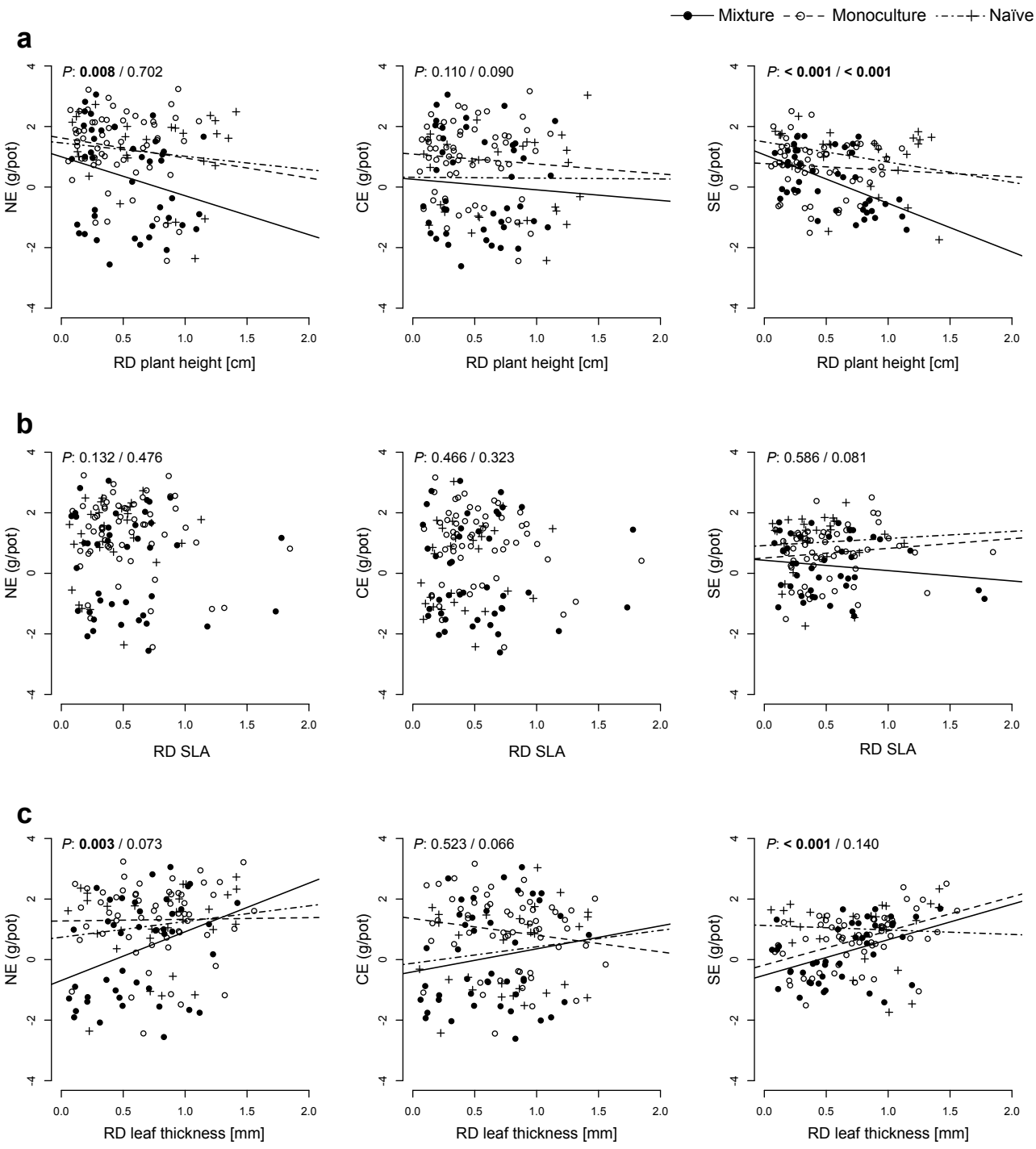


FIG. 4. Biodiversity effects at the first harvest in response to relative differences between species (RDs) for three traits: **a,** plant height (in cm), **b,** specific leaf area (SLA) and **c,** leaf thickness (in mm). Indicated *P*-values refer to ANOVA results for fixed-effects terms from a mixed-effects model with RD, species assembly, selection history and interactions of these as fixed-effects terms and table (including block) as random-effects term: RD / interaction RD × selection history (naïve plants vs. mixture

types vs. monoculture types). Regression lines are plotted in cases for which at least one *P*-value was significant. Left column: NE, middle column: CE, right column: SE.

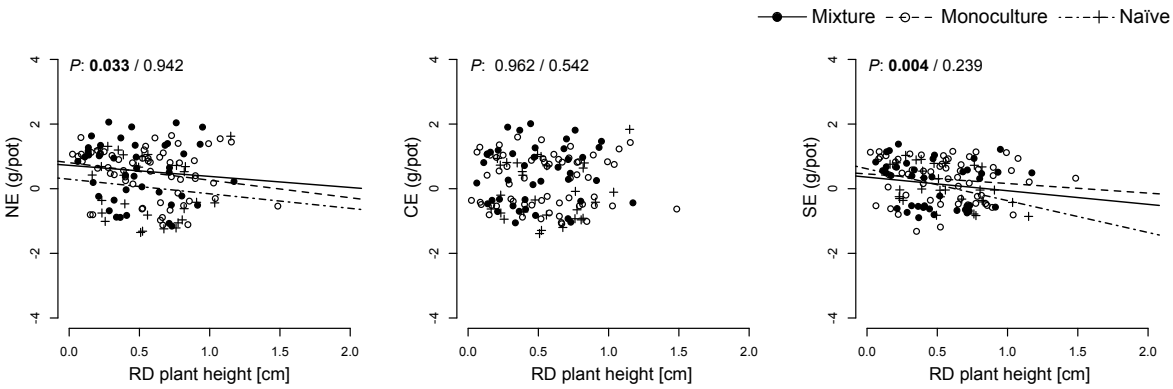


FIG. 5. Biodiversity effects at the second harvest in response to relative differences between species for plant height (in cm). Indicated *P*-values refer to ANOVA results for fixed-effects terms from a mixed-effects model with RD, species assembly, selection history and interactions of these as fixed-effects terms and table (including block) as random-effects term: RD / interaction RD × selection history (naïve plants vs. mixture types vs. monoculture types). Regression lines are plotted in cases for which at least one *P*-value was significant. Left column: NE, middle column: CE, right column: SE.

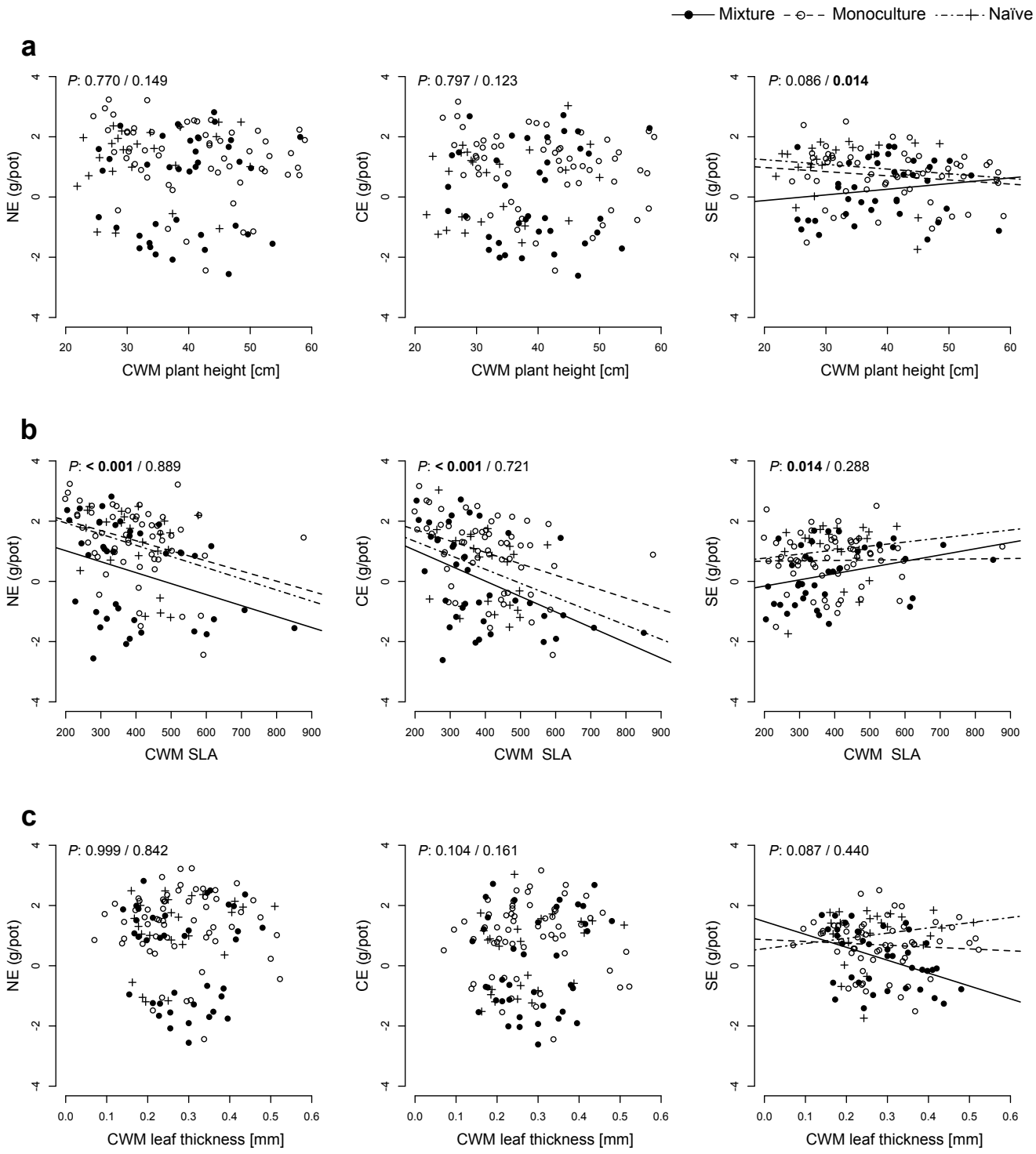


FIG. 6. Biodiversity effects at the first harvest in response to the community-weighted mean (CWM) of three traits: **a,** plant height (in cm), **b,** specific leaf area (SLA) and **c,** leaf thickness (in mm). Indicated *P*-values refer to ANOVA results for fixed-effects terms from a mixed-effects model with CWM, species assembly, selection history and interactions of these as fixed-effects terms and table (including block) as random-effects term: CWM / interaction CWM × selection history (naïve plants vs. mixture types vs. monoculture types). Regression lines are plotted in cases for which at least one *P*-value was significant. Left column: NE, middle column: CE, right column: SE.

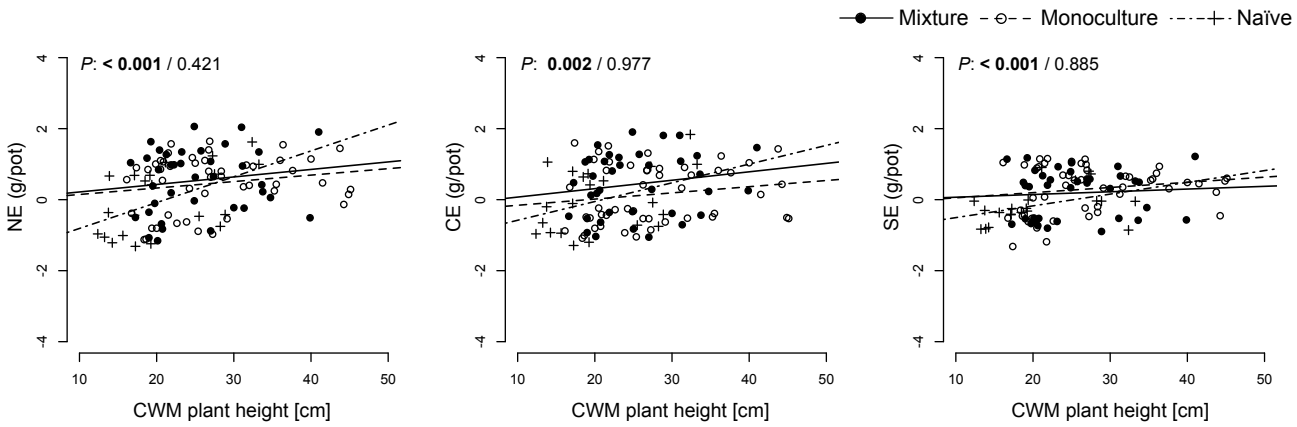


FIG. 7. Biodiversity effects at the second harvest in response to the community-weighted mean (CWM) of plant height (in cm). Indicated *P*-values refer to ANOVA results for fixed-effects terms from a mixed-effects model with CWM, species assembly, selection history and interactions of these as fixed-effects terms and table (including block) as random-effects term: CWM / interaction CWM × selection history (naïve plants vs. mixture types vs. monoculture types). Regression lines are plotted in cases for which at least one *P*-value was significant. Left column: NE, middle column: CE, right column: SE.

TABLE 1. Summary of hypotheses.

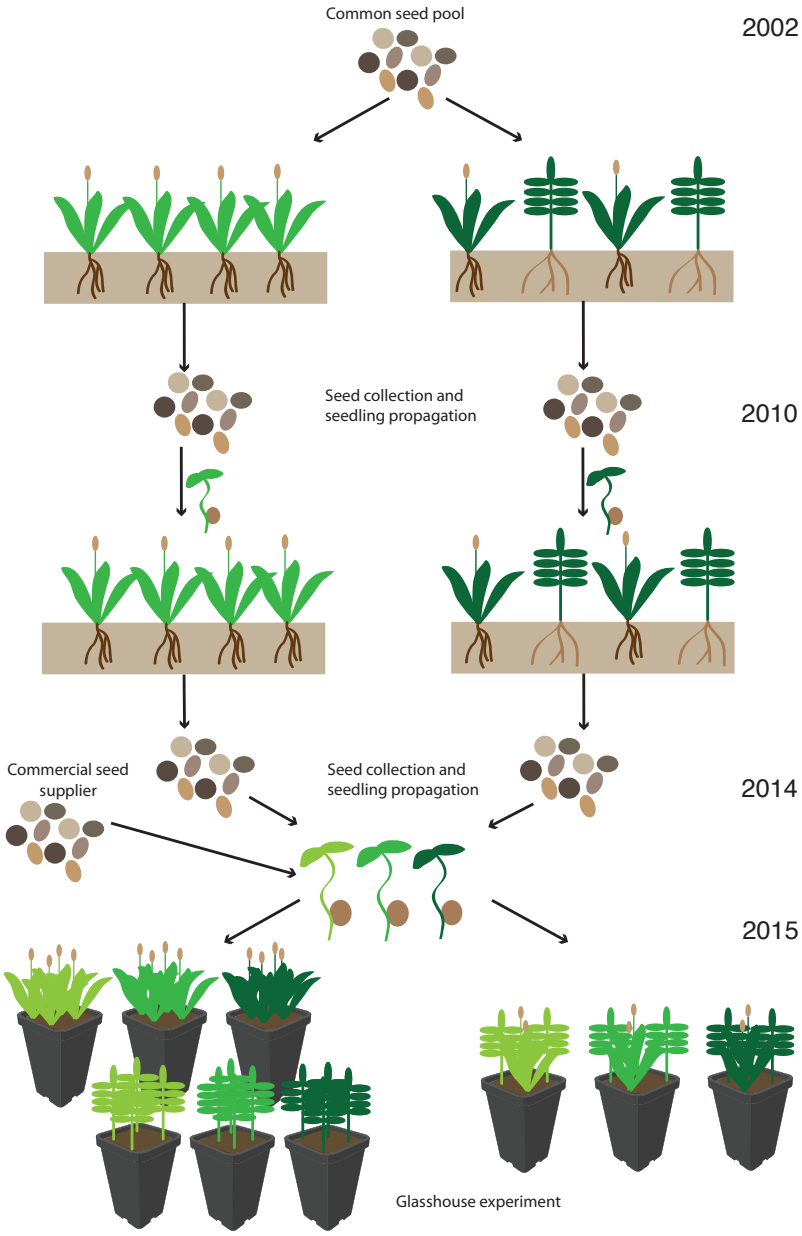
	Hypothesis
1)	Mixture-type plants produce high biomass in mixtures.
2)	Mixture-type plants have large NEs and CEs.
3)	Mixture-type plants show large interspecific trait variation.
4)	Monoculture-type plants produce high biomass in monocultures.
5)	Monoculture-type plants show large intraspecific trait variation.
6)	Large CEs are due to between-species trait variation.
7)	Large SEs are due to large CWMs.

TABLE 2. Results of mixed-effects ANOVA for the aboveground biomass of the test communities.

		Harvest 1			
Source of variation		**nDf**	**dDF**	***F***	***P***
Species assembly:					
	Monoculture vs. mixture	1	173.3	29.09	< 0.001
	Monoculture identity or species combination of mixture	13	171.2	16.53	< 0.001
Selection history:					
	Naïve vs. mono or mix types	1	173	16.63	< 0.001
	Mono vs. mix types	1	169.6	1.78	0.184
Assembly × history:					
	Monoculture vs. mixture × naïve vs. mono or mix types	1	168.4	1.72	0.191
	Monoculture vs. mixture × Mono or mix types	1	172.2	1.69	0.195
	Species assembly × naïve vs. mono or mix types	8	171.7	5.35	< 0.001
	Species assembly × mono types vs. mix types	10	172.3	2.91	0.002
Variance components		**n**	**Var**	**SE**	
	Table (including blocks)	18	1.7512	0.8010	
	Residual (pots)	221	5.8403	0.6395	

		Harvest 2			
Source of variation		**nDf**	**dDF**	***F***	***P***
Species assembly:					
	Monoculture vs. mixture	1	174	10.78	0.001
	Monoculture identity or species combination of mixture	13	171.8	15.47	< 0.001
Selection history:					
	Naïve vs. mono or mix types	1	173.7	42.72	< 0.001
	Mono vs. mix types	1	170.1	5.71	0.018
Assembly × history:					
	Monoculture vs. mixture × naïve vs. mono or mix types	1	168.8	8.56	0.004
	Monoculture vs. mixture × Mono or mix types	1	172.9	3.52	0.062
	Species assembly × naïve vs. mono or mix types	8	172.3	2.15	0.033
	Species assembly × mono types vs. mix types	10	172.9	1.23	0.275
Variance components		**n**	**Var**	**SE**	
	Table (including blocks)	18	0.2451	0.1145	
	Residual (pots)	221	0.9225	0.1009	

Note: nDF = numerator degrees of freedom, dDF = denominator degrees of freedom, *F* = variance ratio, *P* = probability of type-I error. Variance components (Var) and associated standard errors (SE) for the random effects are provided together with the number of replicates.



Appendix S1. The origin of seeds used for the experiment. Seedlings were planted in mixtures and monocultures in Jena in the year 2002. Two sexual reproduction events (in 2010 and in 2014) occurred when seeds were collected and subsequently new seedlings were produced and planted again in the same community composition. Furthermore, seed material purchased from commercial seed suppliers (the same ones providing seed material for the original set up of the Jena Experiment) was included for this study.

ASSEMBLY			SELECTION HISTORY														
			Naïve					Monoculture					Mixture				
			Plantago	*Prunella*	*Veronica*	*Galium*	*Lathyrus*	*Plantago*	*Prunella*	*Veronica*	*Galium*	*Lathyrus*	*Plantago*	*Prunella*	*Veronica*	*Galium*	*Lathyrus*
	Monoculture	*Plantago*	6					6					6				
		Prunella		6					6					6			
		Veronica			6					6					6		
		Galium				6					6					6	
		Lathyrus					0					6					6
	Mixture	*Plantago*															
		Prunella	5					6					6				
		Veronica	0	6				6	6				6	6			
		Galium	0	6	0			6	6	6			6	6	6		
		Lathyrus	6	6	6	0		6	6	6	6		0	0	6	0	

Appendix S2. Experimental design. The full diallel design was intended, however, due to seedling mortality some species assemblies were not feasible (indicated by 0). Each species assembly was replicated six times, except the combination of *Plantago lanceolata* with *Prunella vulgaris* from the naïve selection history, which had five replicates. *Plantago lanceolata*, *Prunella vulgaris* and *Veronica chamaedrys* belong to the functional group small herbs, *Galium mollugo* is a tall herb and *Lathyrus pratense* is a legume.

Appendix S3. Results of mixed-effects ANOVA for biodiversity effects of the test communities at the first harvest.

	NE Harvest 1			
Source of variation	**nDf**	**dDF**	***F***	***P***
Overall mean	1	15.9	26.67	< 0.001
Naïve vs. mono or mix types	1	95.3	0.806	0.372
Mono types vs. mix types	1	93.9	21.01	< 0.001
Species assembly	9	96.7	2.646	0.009
Species assembly × Naïve vs. mono or mix types	4	97.5	4.459	0.002
Species assembly × Mono types vs. mix types	6	98	4.095	0.001
Variance components	**n**	**Var**	**SE**	
Table (including blocks)	18	0.4572	0.2209	
Residual (pots)	221	1.1185	0.1652	

	CE harvest 1			
Source of variation	**nDf**	**dDF**	***F***	***P***
Overall mean	1	15.8	8.214	0.011
Naïve vs. mono or mix types	1	95.9	1.427	0.235
Mono types vs. mix types	1	94.4	14.2	< 0.001
Species assembly	9	97.4	2.534	0.012
Species assembly × Naïve vs. mono or mix types	4	98.3	1.835	0.128
Species assembly × Mono types vs. mix types	6	98.8	2.53	0.025
Variance components	**n**	**Var**	**SE**	
Table (including blocks)	18	0.4415	0.2269	
Residual (pots)	221	1.3254	0.1957	

	SE harvest 1			
Source of variation	**nDf**	**dDF**	***F***	***P***
Overall mean	1	14.2	97.07	< 0.001
Naïve vs. mono or mix types	1	104.2	12.66	0.001
Mono types vs. mix types	1	101.2	10.28	0.002
Species assembly	9	105.5	5.793	< 0.001
Species assembly × Naïve vs. mono or mix types	4	105.9	10.08	< 0.001
Species assembly × Mono types vs. mix types	6	105.9	2.865	0.013
Variance components	**n**	**Var**	**SE**	
Table (including blocks)	18	-0.0005	0.0246	
Residual (pots)	221	0.4927	0.0715	

Note: nDF = numerator degrees of freedom, dDF = denominator degrees of freedom, *F* = variance ratio, *P* = probability of type-I error. Variance components (Var) and associated standard errors (SE) for the random effects are provided together with the number of replicates.

Appendix S4. Results of mixed-effects ANOVA for biodiversity effects of the test communities at the second harvest.

	NE harvest 2			
Source of variation	**nDf**	**dDF**	***F***	***P***
Overall mean	1	15.1	14.35	0.002
Naïve vs. mono or mix types	1	96.1	11.54	< 0.001
Mono types vs. mix types	1	93.6	0.026	0.872
Species assembly	9	97.7	4.837	< 0.001
Species assembly × Naïve vs. mono or mix types	4	98.9	1.463	0.219
Species assembly × Mono types vs. mix types	6	99	1.518	0.180
Variance components	**n**	**Var**	**SE**	
Table (including blocks)	18	0.1390	0.0773	
Residual (pots)	221	0.4958	0.0734	

	CE harvest 2			
Source of variation	**nDf**	**dDF**	***F***	***P***
Overall mean	1	14.5	4.108	0.061
Naïve vs. mono or mix types	1	96.6	5.668	0.019
Mono types vs. mix types	1	93.8	1.524	0.220
Species assembly	9	98.4	1.121	0.356
Species assembly × Naïve vs. mono or mix types	4	99.7	0.584	0.675
Species assembly × Mono types vs. mix types	6	99.8	0.468	0.831
Variance components	**n**	**Var**	**SE**	
Table (including blocks)	18	0.1395	0.0865	
Residual (pots)	221	0.6378	0.0945	

	SE harvest 2			
Source of variation	**nDf**	**dDF**	***F***	***P***
Overall mean	1	15.1	11.66	0.004
Naïve vs. mono or mix types	1	98.8	2.224	0.139
Mono types vs. mix types	1	95.7	2.37	0.127
Species assembly	9	100.8	11.53	< 0.001
Species assembly × Naïve vs. mono or mix types	4	102	3.517	0.010
Species assembly × Mono types vs. mix types	6	101.9	2.541	0.025
Variance components	**n**	**Var**	**SE**	
Table (including blocks)	18	0.0296	0.0223	
Residual (pots)	221	0.2202	0.0324	

Note: nDF = numerator degrees of freedom, dDF = denominator degrees of freedom, *F* = variance ratio, *P* = probability of type-I error. Variance components (Var) and associated standard errors (SE) for the random effects are provided together with the number of replicates.

CHAPTER FOUR

Evidence for rapid genetic divergence in response to community diversity in a grassland biodiversity experiment

Evidence for rapid genetic divergence in response to community diversity in a grassland biodiversity experiment

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B.S., P.V. and S.J.V.M. conceptualized the project; S.J.V.M. carried out the experiment; C.A.M.W. performed the lab work; T.V.G. created the bioinformatics pipelines, M.W.S., S.J.V.M. and B.S. analysed the data; S.J.V.M. and M.W.S. wrote the manuscript with all authors contributing.

Abstract

In long-term grassland biodiversity experiments the positive effect of biodiversity on plant productivity commonly increase with time. Previously it was shown that differential selection in monoculture and mixed-species grassland communities could lead to the rapid emergence of monoculture and mixture phenotypes. Underlying mechanisms for such rapid phenotypic responses are however still unclear. We hypothesize that in biodiversity experiments pre-adapted genotypes or epigenetic variants could be sorted out from the standing genetic or epigenetic variation.

To test if biodiversity acted as a selective environment, we grew offspring from plants that were exposed for twelve years to a monocultures or mixture environment under controlled greenhouse conditions. Using epiGBS, a genotyping by sequencing approach combined with bisulphite conversion to provide integrative genetic and epigenetic data, we showed that plants with a monoculture or mixture background were genetically distinct. Our data reveals a strong correlation between genetic and epigenetic variation and suggest genetic variation as driving force of most epigenetic variation. This pattern was consistently observed across different plant species. These results suggest that, in perennial grassland species, selection of genetic variation underlies the rapid emergence of monoculture and mixture types.

Keywords: biodiversity, epiGBS, epigenetic variation, genetic divergence, grassland species, rapid evolution, representative reduced bisulfite sequencing, selection

Introduction

Biodiversity is crucial for the functioning of a variety of different ecosystems (Hooper et al. 2005). For example, in grasslands, more diverse plant communities were shown to be more productive (Tilman et al. 2001), with stable productivity over time (Allan et al. 2011, Gross et al. 2014, Isbell et al. 2015), and greater resilience towards external perturbations than less diverse communities (Wagg *et al.*, Ecology (in revision), van Moorsel *et al.*, in prep).

The Earth's biosphere is currently challenged by the impacts of anthropogenic environmental change and plant populations may encounter new abiotic or biotic environment due to climate-induced range shifts (Ouborg et al. 2006). The unprecedented rate of environmental change raises the question whether natural communities can adapt fast enough to novel abiotic or biotic conditions. Whereas the influence of environmental factors on adaptive responses of plant populations is well studied (e.g., Schmid 1985, Joshi et al. 2001), much less effort has been devoted to studying the influence of community diversity on population structure and productivity (but see Lipowsky et al. 2011, Kleynhans et al. 2016). In particular, the influence of multi-species interactions for the adaptive response of a species is largely unknown, despite a growing body of evidence pointing towards the importance of species-interaction networks for the maintenance of ecosystem stability (Bastolla et al. 2009). It is conceivable that the feedback between species interactions and their adaptive responses shapes community-level ecosystem functioning.

Whereas in the 1960s it was proposed that there are large differences in the time scales between ecological and evolutionary processes (Slobodkin 1961), it is now well known that micro-evolutionary and ecological processes can occur on the same temporal scale (reviewed by Hairston et al. 2005, Schoener 2011). Thus, it appears that micro-evolutionary processes may allow for an evolutionary rescue in a rapidly changing environment. Understanding how biodiversity, i.e., the interactions between different species, shapes this evolutionary response will be instrumental to anticipate how ecosystems may change in response to global change.

Adaptation depends on several factors. For organisms with a short generation time and asexual reproduction, such as clonal populations of bacteria, mutations and horizontal gene transfer are the main sources of genetic variation (Anderson et al. 2011). However, for species with longer generation times, such as perennial plants, selection far more often acts on standing genetic variation (Barrett and Schluter 2008), resulting in a sorting-out of suitable genotypes (Fakheran et al. 2010). Furthermore, plants may adapt (or better "adjust") to a novel environment by phenotypic plasticity (Price et al. 2003, Turcotte and Levine 2016).

Early indications for phenotypic changes in grassland plant communities were the observed strengthening of biodiversity effects in field biodiversity experiments (Cardinale et al. 2007, Fargione et al. 2007, Reich et al. 2012, Meyer et al. 2016). In other words, an increase in complementarity between species resulted in an increasing positive effect of diversity on productivity over time (e.g. Meyer et al. 2016). These phenotypic changes may be the result of phenotypic plasticity. However, recent

common garden experiments with plant material from different diversity backgrounds (i.e., the Jena biodiversity experiment) give a clear indication for genetic divergence (Zuppinger-Dingley et al. 2014, van Moorsel et al. 2017b), suggesting that natural selection in response to community diversity had previously occurred in the field. In these studies, stronger biodiversity effects (Loreau and Hector 2001) were observed in communities of co-selected plants from a biodiversity experiment (Jena Experiment, see Roscher et al. (2004)) as opposed to communities of plants with a selection history in monocultures (van Moorsel et al. 2017b). The specific community diversity background furthermore altered within- and between-species variation in several plant functional traits (leaf thickness, specific leaf area and plant height), indicating that evolutionary change resulted in decreased or increased complementarity between and within species (van Moorsel et al. 2017b). The emergence of such monoculture and mixed culture types, with different growth performance and plant functional trait variation, thus suggested that community diversity in the field likely acted as a selective environment (Zuppinger-Dingley et al. 2014, Rottstock et al. 2017, van Moorsel et al. 2017b). However, molecular evidence for a genetic divergence between the different populations in the field experiment is still missing.

It should be noted that epigenetics, here defined as meiotically heritable changes in gene expression without changes to the underlying DNA sequence (Verhoeven et al. 2016), has also been proposed to play a role (Bird 2007, Bossdorf et al. 2008, Tilman and Snell-Rood 2014). In a comment accompanying the publication of Zuppinger-Dingley et al. (2014), David Tilman and Emilie Snell-Rood wrote: "[…] laboratory propagation of the plants increased the chance that the differences between the high- and low-diversity selection groups were due to genetic divergence. However, it is possible that epigenetic factors […] could have had a simultaneous role" (Tilman and Snell-Rood 2014). However, the importance of epigenetics in natural populations, and whether it contributes to adaptation, remains elusive (Quadrana and Colot 2016) because it is very difficult to separate epigenetic from genetic variation. An example in which this could be achieved was a study with apomictic clones of *Taraxacum officinale* suggesting that differences in flowering time were mediated by differences in DNA methylation (Wilschut et al. 2016). However, given the fundamental difference between apomixis and sexual reproduction (apomeiosis, parthenogenesis, autonomous endosperm formation), the results from this study may not be directly transferred to non-apomictic plant species.

Here, we tested whether community diversity could act as a selective force leading to the evolution of populations within the same species exhibiting distinct diversity–productivity relationships. In particular, we aimed to establish whether genetic or epigenetic factors were driving the differentiation of plants into mixture types (exhibiting stronger biodiversity effects when planted in mixed-species communities) or monoculture types (exhibiting weaker biodiversity effects when planted in mixed-species communities) within the same species (van Moorsel et al. 2017b). We therefore analyzed genetic and epigenetic variation mixture- and monoculture-type plants in six perennial European grassland species.

Methods and Materials

Plant material

Plant selection histories. To test whether plant types selected over eleven years in mixtures differ genetically or epigenetically from those types selected in monocultures, we chose six species grown in monoculture and mixture plots in the Jena Experiment (Jena, Thuringia, Germany, 51˚N, 11˚E, 135 m a.s.l., see Roscher et al. 2004 for experimental details). The following species belonging to four functional groups were selected: The three small herbs *Plantago lanceolata, Prunella vulgaris* and *Veronica chamaedrys*, the tall herb *Galium mollugo,* and the two legumes *Lathyrus pratensis* and *Onobrychis viciifolia*. For the experiment, plants from three different selection histories were used. Plants without a selection history in the experimental field plots of the Jena Experiment were obtained from commercial seed suppliers (Rieger Hoffmann GmbH, Germany and Otto Hauenstein Samen AG, Switzerland), who also provided the seeds for the original set up of the Jena Experiment in 2002. Plants with a selection history in either mixture or monoculture had been growing in the Jena Experiment since 2002. In 2010, cuttings of these plants were brought to Zurich and used for seed production for an earlier experiment. The propagation of seedlings for this experiment is described elsewhere (Zuppinger et al. 2014). The resulting seedlings were then planted back into the experimental plots in Jena in 2011 in the exact same species compositions from which they originated (for detailed procedure, see van Moorsel *et al.* 2017a).

Seed collection. To collect seed material to be used in the present study, in March 2014 we established plots in an experimental garden in slug-exclosure compartments at the University of Zurich, Switzerland (47°33′N, °37′E, 534 m a.s.l.). For this purpose, we excavated the entire plant communities (0.5 m^2) from some of these experimental plots in Jena by removing blocks of soil including plant vegetation, seedlings and dormant seeds to make sure we transferred the entire plant community. These blocks of soil were then "planted" into 1 m^2 plots, which resulted in larger plots with an identical plant composition to the plots in Jena from which the plants were collected. We added a layer of soil (Gartenhumus, Ricoter, 50% agricultural soil, 50% garden compost) to each plot to make sure the plants established. Netting around each plot minimized the possibility of cross-pollination between the same species from different selection histories. Seeds were collected throughout the growing season of 2014 from monoculture plots and 4- and 8-species mixture plots. The exact community composition of the plots the seeds originated from is listed in Appendix Table S2. Seeds from different mother plants were pooled together. Seeds were cleaned manually for four species and for two species (*Plantago lanceolata* and *Prunella vulgaris*), the seeds were cleaned professionally. The dry seeds were stored at 5° C for cold stratification until germination.

Pot experiment. Seeds were germinated in December 2014 with those from the same species being planted within the same day. Germination was done in germination soil (Ökohum Aussaaterde) under constant conditions in a glasshouse without additional light. Grass seedlings were trimmed to reduce their competitive head start before planting all seedlings in monocultures of four individuals and mixtures of four individuals into pots (2 liter) filled with neutral agricultural soil (Ricoter, 50% agriculture soil, 25% perlite, 25% sand). Seedlings that died in the first 2 weeks were replaced with seedlings of the same age. The experiment was set-up in 6 blocks with each block representing a replicate. Every block contained ca. 80 pots and within each block, pots were placed in the glasshouse in a randomized fashion without reference to selection history or species assembly. Single pots always contained four plants of a single selection history. In total, we planted 36 monocultures and 81 mixtures from mixture history, 48 monocultures and 159 mixtures from monoculture history and 33 monocultures and 100 mixtures from seedlings without a common selection history. Every species combination was replicated, if possible, six times for each selection history (resulting in 457 pots and 1828 plants). During the experiment, plants were watered according to demand and grown at constant temperatures (17–20°C during the day, 13–17° during the night) with no additional light added. The plants were not fertilized. Due to an infestation of white flies and spider mites, the insecticide SanoPlant Neem (1% Azadirachtin A (10 g/l); Maag AG) was applied three times. Against powdery mildew the fungicide Fenicur (*Oleum foeniculi*, Andermatt Biocontrol) was applied twice.

Sampling. Samples for subsequent epigenetic and genetic analysis were harvested in May 2015, after twelve weeks of growth in the greenhouse. In each pot, all four plants were sampled. One young leaf per plant was cut from the living plant and immediately shock-frozen in liquid nitrogen. The samples were then stored at –80°C before shipment to the Netherlands for further processing.

Genetic analysis

We measured both genetic and epigenetic variation in plants from monocultures and mixtures when propagated in monocultures and mixed communities using a novel reference-free bisulfite method (van Gurp et al. 2016). This method allows to measure DNA cytosine methylation levels and identify single nucleotide polymorphisms with merely one lane of sequencing per 96 samples.

Sample procession and library preparation. The epiGBS protocol used is a further-developed protocol based on the protocol of van Gurp *et al.* (van Gurp et al. 2016). The main improvements are on the enzyme combination used and the use of a "wobble" adapter that facilitates the computational removal of PCR duplicates and a conversion-control nucleotide that also allows easier Watson / Crick identification.

Description of samples. For 348 samples, (Csp6I/NsiI) epiGBS libraries were created and sequenced over 4 Hiseq 2500 lanes. These samples were divided over six species and three selection histories (see Appendix table S1).

DNA extraction. Plant material was disrupted by bead-beating frozen leaf tissue in a 2 mL eppendorf tube with 2–3 mm stainless steel beads. No more than 100 mg of fresh tissue was used per sample. DNA isolation was performed using the NucleoSpin® 8 Plant II Core Kit (740669.5 Macherey Nagel). We followed the manufacturers protocol with the following modifications. Cell lysis was done using Cell lysis buffer PL1 for 30 instead of 10 min. After lysis and initial centrifugation, the lysate was pipetted to fresh 2.5 mL tubes, avoiding the cell debris. An extra centrifugation of 5 min at 18.000 g step was done and the lysate transferred to a 96-well rack for the next steps. At the step where the washed columns were dried, we centrifuged 5 min at 4800 g to get rid of the last remaining wash buffer. DNA concentration was determined using Qubit® 2.0 Fluorometric dsDNA HS Assay Kit (Q32851 Life technologies).

DNA digestion. Per individual, 30–300 ng gDNA was digested overnight (17 hrs) at 37° C in a volume of 40 µL containing 1x FD buffer (Thermo Scientific), and 2 uL of both *Csp*6I (FD0214, Thermo Scientific) and *Nsi*I (R0127S, NEB).

Adapter ligation. Following digestion, barcoded "wobble" adapters were ligated to the fragments (Appendix Figure S2). To minimize the possibility of misidentifying samples as a result of sequencing or adapter synthesis error, all pair-wise combinations of barcodes differed by a minimum of three mutational steps. Barcode lengths were modulated from 4 bp to 6 bp to maximize the balance of the bases at each position in the overall set. For the ligation, 4 uL of a sample specific barcode combination of both BA and CO adapters (600 pg/uL), 6 uL T4 DNA ligase buffer, 1uL T4 DNA ligase (M0202M, NEB) and 5 uL of distilled water were added to the digestion mix to a total volume of 60 uL. Ligation was performed for 3 hrs at 22° C followed by 4° C overnight.

Pooling and cleanup. In order to assess the quality of libraries, the pooling was done per species in batches of around 12 samples per pool. When pooled, the total volume of the pool was reduced by Qiaquick PCR cleanup (28104, Qiagen) to 40 µL. The libraries were size-selected by a 0.8x Agencourt AMPure XP (A63880, Beckman coulter) purification favouring > 200 bp DNA fragments and eluted in a total volume of 22 µL.

Nick translation. To prevent the formation of adapter dimers, the barcoded adapters were not phosphorylated. Therefore, after the ligation, each DNA fragment–adapter connection was nicked at the position of the yellow dots (Appendix Figure S2). This

nick is repaired by nick translation that recreates the total non-(5mC) methylated adapter strand and during that process also "unwobbles" the adapters since the removed nucleotides are replaced by complementing nucleotides. This nick repair prevents the partial loss of the adapter during bisulfite treatment. The nick translation reaction (1 hour at 15° C) was performed in a reaction of 25 µL containing 19.25 µL of the purified library, 2.5 uL of 10 mM 5-methylcytosine dNTP Mix (D1030 Zymo research), 2.5 uL NEBuffer 2 and 0.75 uL DNA polymerase I (M0209, NEB).

Bisulphite conversion. For bisulfite conversion of non-methylated cytosines, 20 µL of the nick-translated library was used. Bisulfite treatment was performed using the EZ DNA Methylation-Lightning™ Kit (Zymo Research) with the following program according to the manufacturers protocol: 8 min at 98° C, 1 hour at 54 °C followed by up to 20 h at 4° C.

epiGBS PCR. Library amplification was done in four individual 10-µL reactions containing 1 µL ssDNA template, 5 µL KAPA HiFi HotStart Uracil+ ReadyMix (Kapabiosystems), 3 pmol of each illumina PE PCR Primer (5'-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-3' and 5'-CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCT-3'). Temperature cycling consisted of 95° C for 3 min followed by 18 cycles of 98° C for 10 s, 65° C for 15 s, 72° C for 15 s with a final extension step at 72° C for 5 min. Replicate PCR products were pooled and quantified using a Qubit® dsDNA HS Assay Kit (Life technologies). The quality of the Libraries was assessed by analyzing 1 µL on a High Sensitivity DNA chip on a 2100 Bioanalyzer system (Agilent). Libraries were considered suitable for sequencing if the majority of DNA fragments were between 150-400 bp. When the libraries passed quality control, they were pooled according to concentration and number of samples in the species pool, so that each individual sample was expected to yield an equal number of clusters on the Illumina flow cell. Before sequencing, the libraries were spiked with 10% PhiX control to increase the complexity of the libraries.

Sequencing. Finally, Paired-End sequencing was performed on a Hiseq2500 sequencer using the HiSeq v4 reagents and the latest version of the HiSeq Control Software (v2.2.38), which optimizes the sequencing of low-diversity libraries (http://res.illumina.com/documents/products/technotes/technote-hiseq-low-diversity.pdf). As the first five cycles of a sequencing run are used to calculate the color matrix, our barcode design achieves almost perfect balance of the first five nucleotides when equal numbers of sequences are obtained per "A" barcode. The "B" barcodes do not have this requirement; hence same-length barcodes were used.

Statistical analysis

Data processing. De-multiplexing, *de novo* reference construction, trimming, alignment, strand-specific variant calling, and methylation calling were done for each species as described (van Gurp et al. 2016). *De novo* reference sequences were annotated with DIAMOND (protein coding genes; NCBI non-redundant proteins as reference; version 0.8.22; Buchfink, Xie, and Huson 2015) and RepeatMasker (transposons and repeats; *Embryophyta* as reference "species"; version 4.0.6; Smit, Hubley, and Green 2013–2015). We summarized the transposable element and repeat classes into “transposons” comprising DNA, LTR, LINE, SINE, and RC transposon, and “repeats” including satellite, telomeric satellite, simple, rRNA, snRNA, unknown, and unclassified repeats. The annotation was then used to classify the genetic and epigenetic variants into the different feature contexts (e.g., to identify whether a single nucleotide polymorphism is located in a gene or a transposon).

Genetic variation: visualization of genetic distances with single nucleotide polymorphisms (SNPs). For each species, we initially filtered the genetic variation data for single nucleotide polymorphisms (SNPs) sequenced in at least three individuals per population (i.e., experimental group) with a total coverage between 5 and 100. Individuals with a SNP calling rate below 30% were removed from the analysis of genetic variation ("pool_pla_lan_15", "pool_pla_lan_46", "pla_lan_81", "pla_lan_82", "pla_lan_52", "pla_lan_83", "pla_lan_95", "pla_lan_111", "pru_vul_60", "pru_vul_79", "pru_vul_80", "pru_vul_87", "pool_pru_vul_22", "pool_pru_vul_24", "ono_vic_15", and "ver_cha_73"). Data were then filtered for SNPs sequenced in all remaining individuals with a total coverage between 5 and 100. SNP allele frequencies were scaled with the function “scaleGen” from adegenet (version 2.0.1; Jombart 2008) and genetic distances between the individuals were visualized with t-SNE (van der Maaten and Hinton 2008, van Der Maaten 2014). To select the SNPs with the highest differentiation between the populations, we calculated Jost's D (Jost 2008) with the function “basic.stats” from hierfstat (version 0.04-22; Goudet and Jombart) and only included the top 5% in the visualization.

Genetic variation: test for genetic differentiation between populations with single nucleotide polymorphisms (SNPs). SNP data were processed and filtered as described before. To test for genetic differentiation between populations with different selection histories and of different assemblages, we used a hierarchical model with the factor assembly being nested within the factor selection history and the functions “test.within” and “test.between” from hierfstat (version 0.04-22; Goudet and Jombart) as described (Meeûs and Goudet 2007). This analysis was carried out with the (1) entire data set, (2) SNPs located within genes, and (3) SNPs located within transposons. If more than 1000 SNPs were available, the analysis was done with 1000 randomly selected SNPs. Overall hierarchical *F*-statistics were calculated with the function “varcomp.glob” from hierfstat (version 0.04-22; Goudet and Jombart) but using all SNPs available within genes or transposons.

Epigenetic variation: identification of differentially methylated cytosines (DMCs). For each species, we filtered the epigenetic variation data for cytosines sequenced in at least three individuals per population (i.e., experimental group) with a total coverage between 5 and 200. Variation in percent DNA methylation at each individual cytosine was then analyzed with a linear model in R (R Core group 2016) according to a crossed factorial design with the two explanatory factors "selection history" (mixture vs. monoculture vs. none) and "current assembly" (monoculture vs. 2-species mixture), and the interaction between them (except for *O. viciifolia*). To avoid underestimation of variation, and loss of type-I error control in cases where fitted values of one or more population were close to 0% or 100%, we employed the formula for reduced residual degrees of freedom (Lun and Smyth 2017). *P*-values for each model term were adjusted for multiple testing to reflect false discovery rates (FDR). A cytosine was defined as differentially methylated (DMC) if the FDR was below 0.01 for any of the model terms. Percent DNA methylation of these DMCs was then used to visualize the epigenetic distances between the individuals with t-SNE (van der Maaten and Hinton 2008, van Der Maaten 2014). Missing data were imputed using the mean of the 5 to 7 nearest neighbors (number of neighbors was sequentially increased during imputation).

Results

Visualization of genetic distances between the plant individuals using 5 % of the loci with the strongest divergence between the populations clearly separated the individuals according to their population of origin (i.e., selection history) in five out of six species (Fig. 2). The separation for *P. lanceolata* was incomplete, with individuals originating from the individual seed supplier ("none") being interspersed in the other populations. Individuals from the monoculture- and mixed-culture selection history mostly separated, but there was an outgroup with individuals from all selection histories. It is possible that these individuals originate from a different subpopulation present in the original seed pool compared to the other individuals (i.e., that the original seed pool consisted of at least two distinct populations). In addition to the separation by the selection history, individuals (in particular from *P. lanceolata*) also minimally clustered according to the current diversity level (i.e., assembly). However, this was not surprising given that the distances were visualized with the most divergent loci between the populations (i.e., all combined levels of the factors selection history and assembly).

Considering that the plants were assigned randomly to the current diversity level (i.e., assembly treatment), we expected a genetic differentiation according to the selection history but not the assembly. We therefore tested for a significant genetic divergence between the selection histories and the assemblies using hierarchical *F*-statistics (Table 1, de Meeûs and Goudet 2007). As expected, divergence between assemblies was rarely significant. In contrast, genetic differentiation between the selection histories was always significant in four out of six species. Exceptions were

O. viciifolia and *P. lanceolata*, for which the genetic differentiation between selection histories was always marginally insignificant. For *O. viciifolia*, SNPs located in transposons differed significantly between the assemblies (P = 0.046). However, overall SNPs and SNPs located in genes did not show a significant genetic differentiation between the assemblies ($P_{overall}$ = 0.252, P_{genes} = 0.203). In case of *P. lanceolata*, overall SNPs differed significantly between the assemblies ($P_{overall}$ = 0.007), but SNPs located in either genes or transposons were marginally insignificant (P_{genes} = 0.068 and $P_{transposons}$ = 0.073). In summary, *P. vulgaris* and *V. chamaedrys* exhibited the strongest and most significant differentiation according to the selection histories and clearly no differentiation between the assemblies.

Variation in DNA methylation levels at individual cytosines was on average significant (FDR < 0.01) at 0.2 % of all cytosines tested (Table 2). Differences were mostly limited to the selection histories and rare between the assemblies. Exceptions were *L. pratensis* and *P. lanceolata*, for which the differences between selection histories and assemblies were similar in terms of the number of differentially methylated cytosines (DMCs). However, for these species, differences were overall very rare (0.01 % and 0.08 % of all tested cytosines were significant in *L. pratensis* and *P. lanceolata*, respectively). Likewise, visualization of epigenetic distances between the plant individuals using DMCs did not separate the individuals of *L. pratensis* and *P. lanceolata* into their populations. This may indicate that the differences found within these species were false positives. However, the individuals of the remaining species were well clustered according to their selection history. In summary, differences in DNA methylation were limited and likely exclusively between the different selection histories.

Discussion

In a glasshouse experiment we observed stronger biodiversity effects for plant communities consisting of plants selected from plant mixtures, compared to monocultures (van Moorsel et al., 2017). Here, we aimed to find the mechanisms underlying this phenotypic variation. Plants are known to adapt very fast to their environment by phenotypic plasticity, for example by modifying leaf architecture in response to a change in light availability. Due to the common garden nature of our experiment, however, we could rule out that merely phenotypic plasticity was responsible for our observations and we expected a genetic signal. A rapid genetic divergence within 15 years has previously been observed in two plant species exposed to a change in climatic conditions (Ravenscroft et al. 2015). Here we observed a similar rapid genetic divergence, but in response to community diversity.

All six species clustered more clearly according to their genetic origin compared to the assembly in the pot experiment, which was expected. The most interesting species to discuss is *P. vulgaris*, because it is the only species for which plant individuals from each selection history were grown in both assembly types. Plant individuals of *P. vulgaris* were also in particular influenced by their selective past (see Chapter 3). For epigenetic and genetic distances, we observed that not only

did the three selection histories cluster, but that the "none" history was more genetically distant than the "monoculture" and "mixture" selection histories. This is likely because we purchased the plant material for the "none" history at a different time point (in 2014), compared to the seeds that were used in the original set up of the Jena Experiment in 2002. For *P. vulgaris*, epigenetic and genetic distances showed a very similar pattern, highlighting a tight correlation between genetic and epigenetic variation. For *V. chamaedrys, G. mollugo* and *L. pratensis*, the clustering followed a similar pattern observed for *P. vulgaris*, but the "none" history was genetically less distant. For other species, the results are less straightforward. *P. lanceolata* and *L. pratensis* interestingly cluster according to the genetic signal, but not according to DMCs. This indicates that epigenetic variation can also manifest itself without a strong correlation to the underlying genetic variation.

A special case is *O. viciifolia*. Even though we transplanted the entire subplot from the experimental field site in Jena to the experimental plots in Zurich, in this case only one maternal individual each was left in monoculture and in mixture selection history. Genetic differences between the offspring of these maternal individuals were expected because *O. viciifolia* is an outbreeding species.

Another limitation in this study is the fact that we had only one plot per diversity level per species. Therefore, our results do not only reflect an adaptation to specific community diversities (mixture vs. monoculture), but also to specific community compositions. Nevertheless, our study provides evidence for rapid evolution in non-model grassland species from a biodiversity field experiment, probably due to a sorting out from standing genetic variation (Fakheran et al. 2010).

To conclude, our results show that, within a species, plants could be classified on either monoculture or mixture selection history based on their single nucleotide polymorphisms (SNPs) in a representative part of the genome. Epigenetic variation between individuals (DMCs) of the same species was correlated to underlying genetic variation, indicating that, in the grassland species that we tested, it may be a genetic signal that drives the rapid emergence of monoculture and mixture sub-types.

Our findings suggest that selection on standing genetic variation is a powerful driver of evolution even in the absence of many generations of plant growth. In addition, we propose that community diversity had the selective power to differentiate plant populations within species into mixture and monoculture sub-types within only a few years. Molecular tools and the integration of evolutionary concepts into plant community ecology can open up a whole new alley of exciting research, which should be exploited in order to understand the community evolutive processes (Shafer et al. 2015) that lead to the plant community compositions and structures as we see them today.

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Acknowledgements

We thank T. Zwimpfer, M. Furler, D. Trujillo and D. Topalovic for technical assistance. This study was supported by the Swiss National Science Foundation (grants number 147092 and 166457 to B. Schmid) and the University Research Priority Program Global Change and Biodiversity of the University of Zurich. S.J.V.M. was furthermore supported by a travel grant from the Congenomics network.

Author contributions

S.J.V.M, P.V. and B.S. conceptualized the study, S.J.V.M. carried out the pot experiment and collected plant material, C.A.M.W. performed the lab work and created the sequencing library and T.V.G. created the bioinformatics pipeline. M.W.S. and S.J.V.M. analyzed the data and wrote the first draft of the manuscript. All authors contributed substantially to revisions.

Supporting information

Additional supporting information may be found in the online version of this paper.

Figure 1. Schematic representation of the glasshouse experiment. Test monocultures and 2- species mixtures were assembled with either plants from mixture background (green), plants with a background in monoculture experimental plots (orange) or plants originating from seeds purchased from a commercial seed supplier (blue).

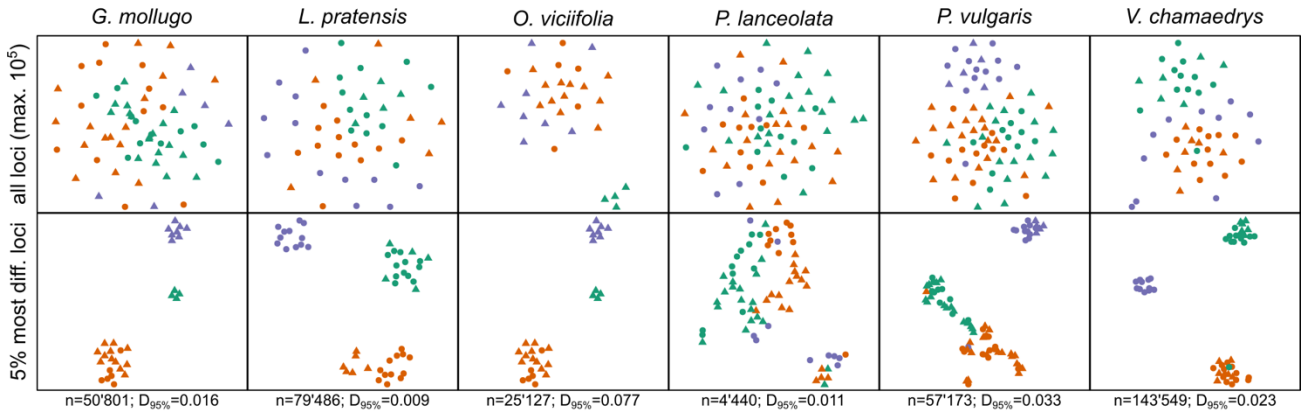

Figure 2. Genetic distance between individuals of the different populations for the six species. Green: selection history in mixture, orange: selection history in monocultures, blue: no history. Triangles: current assembly monoculture, circles: current assembly mixture. Top row: all loci included in analyses, bottom row: only 5% most differentiated loci between selection histories included.

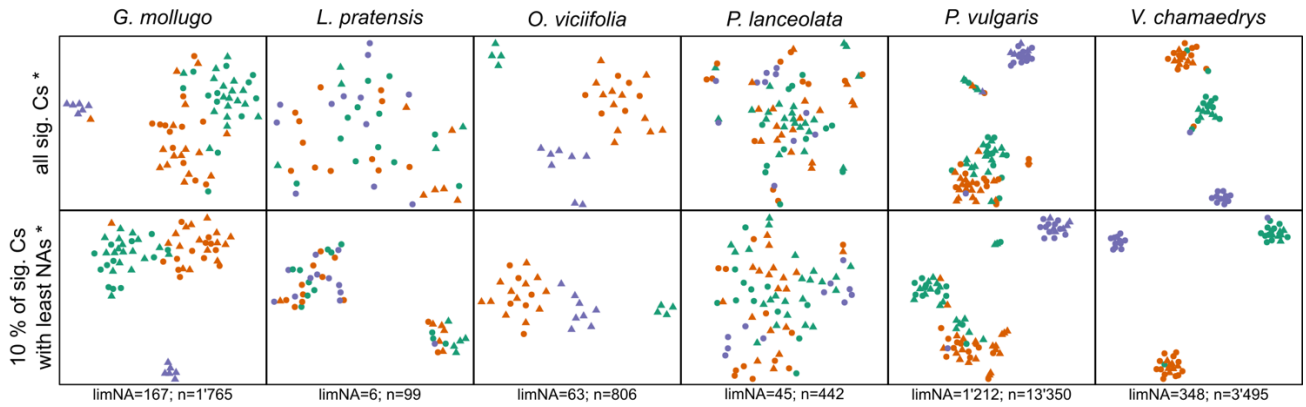

Figure 3. Epigenetic distance between individuals of the different populations for the six species. Green: selection history in mixture, orange: selection history in monocultures, blue: no history. Triangles: current assembly monoculture, circles: current assembly mixture. Cytosines with significant differences in DNA methylation (FDR < 0.01) are shown in the top row. In the bottom, we show these cytosines with the least NAs (the 10% most complete). NAs were imputed with 5-7 nearest neighbors (irrespective of the selection history or assembly).

Table 1. Results of a nested model to test for genetic differentiation. Shown are number of references and number of SNPs in genes and transposons or only genes and only transposons for each of the six species tested. *F*-values are fixation indices corresponding to 1-heterozygosity$_{observed}$/heterozygosity$_{expected}$ of a (sub-)population.

Species and Genomic Feature	Number of References	Number of SNPs	$P_{History}$	$P_{Assembly\ within\ History}$	$F_{History}$	$F_{Assembly\ within\ History}$	$F_{Individual}$
G. mollugo	740940	50780	0.039	0.114	0.029	0.003	-0.160
only genes	82035	13627	0.039	0.056	0.028	0.002	-0.177
only transposons	61127	8801	0.039	0.097	0.026	0.002	-0.172
P. lanceolata	560171	4440	0.039	0.007	0.003	0.007	-0.085
only genes	58529	684	0.032	0.068	0.005	0.004	-0.106
only transposons	38805	622	0.039	0.073	0.003	0.003	-0.216
L. pratensis	703364	79486	0.079	0.062	0.008	-0.001	-0.100
only genes	101477	17899	0.102	0.2	0.006	-0.001	-0.119
only transposons	131964	20401	0.055	0.883	0.006	-0.001	-0.112
P. vulgaris	357406	57173	0.005	0.628	0.085	0.001	-0.167
only genes	46084	6038	0.005	0.831	0.059	0.001	-0.297
only transposons	32108	5532	0.005	0.477	0.060	0.002	-0.287
O. viciifolia	429316	25127	0.082	0.252	0.086	-0.006	-0.174
only genes	57927	7593	0.082	0.203	0.073	-0.005	-0.202
only transposons	45669	6319	0.082	0.046	0.073	-0.002	-0.202
V. chamaedrys	599411	143549	0.039	0.328	0.046	0.002	-0.230
only genes	63783	24087	0.039	0.547	0.039	0.002	-0.248
only transposons	41800	16172	0.039	0.281	0.040	0.001	-0.263

Note: "History" refers to selection history ("mixture" vs. "monoculture" vs. "none" and "Assembly" refers to "mixture" vs. "monoculture" test assemblies in the glasshouse pot experiment. Only SNPs without NA's were included.

Table 2. Number of cytosines with significant differences (FDR < 0.01) in DNA methylation between selection-history treatments and assemblies.

Model term	*G. mollugo*	*L. pratensis*	*O. viciifolia*	*P. lanceolata*	*P. vulgaris*	*V. chamaedrys*
Selection history (H)	1740	34	794	121	12245	3287
Assembly (A)	59	62	20	154	185	157
H × A	72	81	NA	307	1707	352
Any	1765	99	806	442	13350	3495
Tested	1152554	702001	519496	546962	2388577	2175490
% DMCs	0.1531	0.0141	0.1552	0.0808	0.5589	0.1607

Note: P. vulgaris is the only species for which plant individuals from each selection history were grown in both assembly types. For all other species, the selection history "none" was only present in either monocultures (*L. pratensis*, *P. lanceolata*, *V. chamaedrys*) or mixtures (*G. mollugo*, *O. viciifolia*). For *O. viciifolia*, plant individuals from the selection history "mixture" were present only in mixtures.

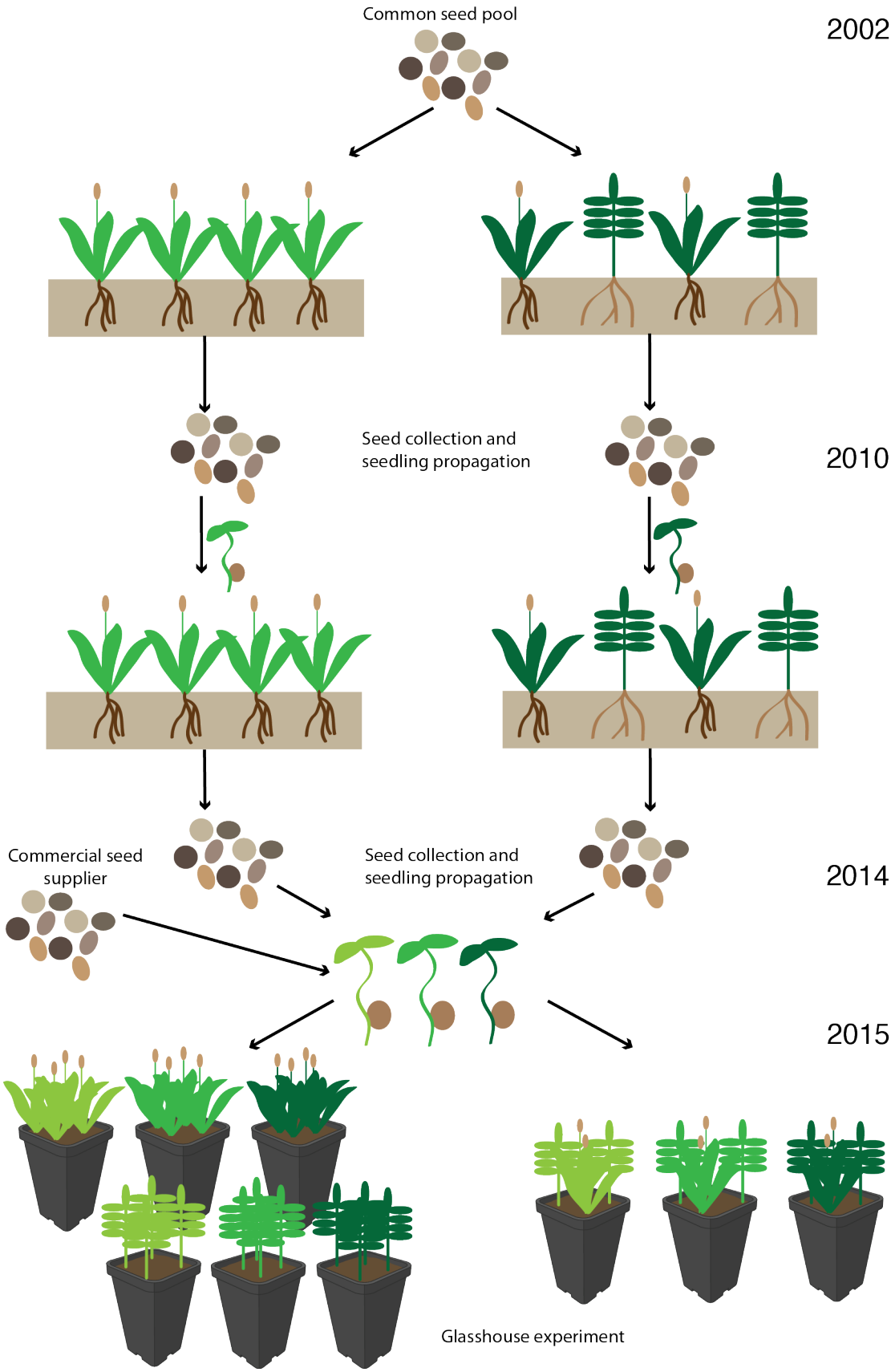
Common seed pool
2002
Seed collection and
seedling propagation
2010
Commercial seed
supplier
Seed collection and
seedling propagation
2014
2015
Glasshouse experiment

Fig. S1. The origin of seeds used for the glasshouse experiment and genetic analysis. Seedlings were planted in mixtures and monocultures in Jena in the year 2002. Two reproduction events occurred when seeds were collected and subsequently new seedlings were produced and planted again in the same community composition.

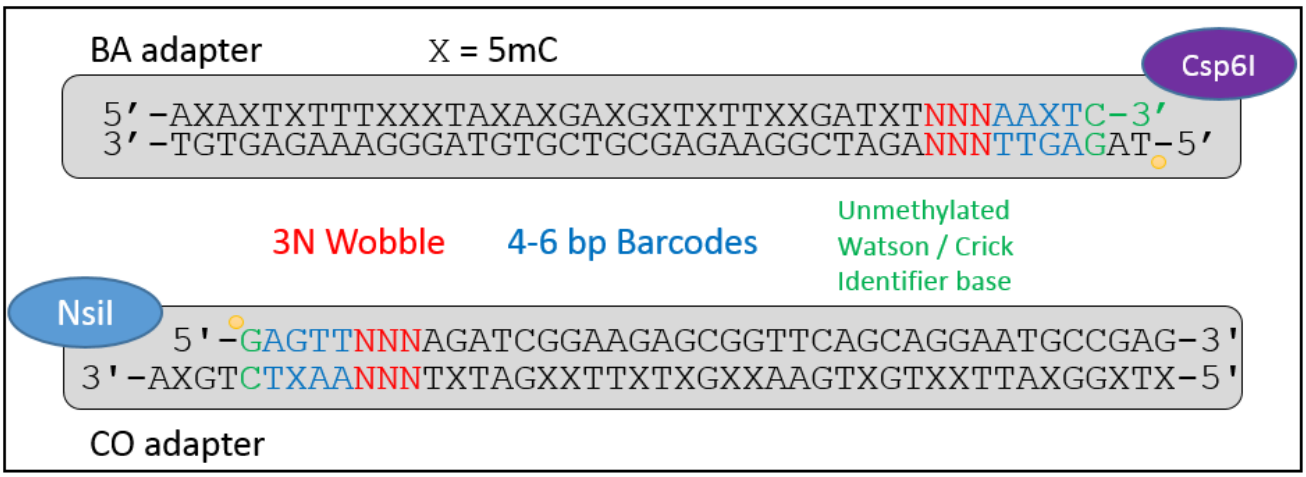

Fig. S2. Adapter overview. Each DNA fragment – adapter connection was nicked at the position of the yellow dots.

Table S1. Sample overview**.**

	History	Monoculture			
	Pilot	Run 1	Run 2	Run 3	Total
Gm	6	8	8	6	28
Lp	6	6	6	0	18
Pl	6	12	12	0	30
Pv	6	9	9	8	32
Vc	6	7	6	0	19
Ov	0	0	0	19	19
	30	42	41	33	146

	History	Mixture			
	Pilot	Run 1	Run 2	Run 3	Total
Gm	6	9	9	6	30
Lp	6	6	5	0	17
Pl	6	12	14	3	35
Pv	6	9	9	6	30
Vc	6	6	6	0	18
Ov	0	0	0	4	4
	30	42	43	19	134

	History	None			
	Pilot	Run 1	Run 2	Run 3	Total
Gm	0	0	0	6	6
Lp	0	3	3	6	12
Pl	0	3	3	6	12
Pv	0	3	3	12	18
Vc	0	3	3	6	12
Ov	0	0	0	8	8
	0	12	12	44	68

	All
	Total
Gm	64
Lp	47
Pl	77
Pv	80
Vc	49
Ov	31
	348

Total	60	96	96	96	**348**

Note: *Galium mollugo*, Lp: *Lathyrus pratensis*, Pl: *Plantago lanceolata,* Pv: *Prunella vulgaris*, Vc: *Veronica chamaedrys*, Ov: *Onobrychis viciifolia*.

Table S2. Community diversity and composition of the plots the seeds originated from**.**

Plot in Jena	Community diversity	Number of species	Target species	Plot community
B3A01	Monoculture	1	*Galium mollugo*	*Galium mollugo*
B2A21	Mix FG-Mixture	8	*Galium mollugo*	*Crepis biennis, Galium mollugo, Onobrychis viciifolia, Leontodon hispidus, Plantago media, Sanguisorba officinalis, Lotus cornyculatus*
B2A04	Monoculture	1	*Geranium pratense*	*Geranium pratense*
B2A12	Mono FG-Mixture	8	*Geranium pratense*	*Geranium pratense, Knautia arvensis, Galium mollugo, Leucanthemum vulgare, Anthriscus sylvestris, Ranunculus acris, Heracleum sphondylium, Sanguisorba officinalis*
B3A12	Monoculture	1	*Lathyrus pratensis*	*Lathyrus pratensis*
B1A12	Mono FG-Mixture	8	*Lathyrus pratensis*	*Lathyrus pratensis, Trifolium campestre, Trifolium dubium, Trifolium fragiferum, Trifolium hybridum, Medicago lupulina, Medicago varia, Onobrychis viciifolia*
B2A15	Monoculture	1	*Onobrychis viciifolia*	*Onobrychis viciifolia*
B2A21	Mix FG-Mixture	8	*Onobrychis viciifolia*	*Crepis biennis, Galium mollugo, Onobrychis viciifolia, Leontodon hispidus, Plantago media, Sanguisorba officinalis, Lotus corniculatus*
B2A13	Monoculture	1	*Plantago lanceolata*	*Plantago lanceolata*
B1A14	Mix FG-Mixture	8	*Plantago lanceolata*	*Plantago lanceolata, Anthriscus sylvestris, Daucus carota, Leontodon hispidus, Luzula campestris, Trifolium campestre, Trifolium fragiferum, Trisetum flavescens*
B1A18	Monoculture	1	*Prunella vulgaris*	*Prunella vulgaris*
B2A01	Mix FG-Mixture	4	*Prunella vulgaris*	*Prunella vulgaris, Knautia arvensis, Trifolium pratense, Anthoxanthum odoratum*
B3A17	Monoculture	1	*Veronica chamaedrys*	*Veronica chamaedrys*
B1A03	Mix FG-Mixture	8	*Veronica chamaedrys*	*Veronica chamaedrys, Cyn cri, Glechoma hederacea, Lotus cornyculatus, Medicago lupulina, Phleum pratense, Primula veris, Trisetum flavescens*

Note: FG = functional group. The four functional groups are: legumes, tall herbs, small herbs and grasses.

CHAPTER FIVE

Testing for co-adaptation of plants and arbuscular mycorrhizal fungi in a biodiversity experiment

Testing for co-adaptation of plants and arbuscular mycorrhizal fungi in a biodiversity experiment

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B.S., T.H., D.Z.D. and S.J.V.M. conceptualized the project; T.H. and S.J.V.M. carried out the experiment; M.W.S., T.H. and B.S. analysed the data; T.H. and S.J.V.M. wrote the first draft of the manuscript. All authors contributed to the final manuscript.

Summary

- Interactions between plants and arbuscular mycorrhizal fungi (AMF) have received much attention but evidence for potential co-adaptation of plants and AMF in the course of ecological experiments is scarce. It was shown, however, that plants selected in monocultures for eight years evolved positive plant–soil feedbacks, potentially due to co-adaptation with AMF and increased pathogen defence.
- We tested co-adaptation with AMF as main hypothesis and increased pathogen defence as secondary hypothesis. We conducted a glasshouse plant–soil feedback experiment using seven grassland species selected over 12 years in monocultures or mixtures. Plants were grown in sterile soil, which was inoculated with either AMF from plant monocultures, AMF from plant mixtures or with a positive control (*Rhizoglomus irregulare*).
- We found mixed evidence for co-adaptation between monoculture-type plants and monoculture AMF and between mixture-type plants and mixture AMF and often co-adaptation was detrimental rather than beneficial for the plants. The secondary hypothesis was more clearly supported as monoculture-type plants suffered less damage from aboveground pathogens.
- We show that co-adaptation between plants and AMF can occur over time but responses to selection in plant monocultures versus mixtures strongly differ between plant functional groups and within them between plant species.

Key-words: growth–defence trade-off, plant–AMF co-adaptation, selection, plant–soil feedbacks, rapid evolution

Introduction

Associations between plants and soil organisms have received much attention in the past decades (e.g. Bever *et al.*, 1997; Klironomos, 2002; van der Heijden *et al.*, 2006; Van Nuland *et al.*, 2016) but studies have not often considered that interactions between plants and such organisms may change over ecological time-scales through adaptation (Lekberg & Koide 2014). In particular, it has not been tested if the co-adaptation of plants and beneficial soil organisms depends on local plant diversity. In monocultures plants are exposed to a stronger accumulation of specialized pathogens than in diverse plant communities, thus potentially reducing productivity of monocultures over time (Kulmatiski, Beard & Heavilin 2012b; van der Putten *et al.* 2013b; Marquard *et al.* 2013). A recent study found that plants in monocultures evolved positive and plants in mixture evolved negative plant–soil feedbacks after eight years of selection in the respective communities (Zuppinger-Dingley et al. 2016). The selection pressure driving this rapid evolutionary change may have been either 2) the presence of beneficial soil organisms which could lead to increased mutualistic interactions with plants via co-adaptation or/and 2) a greater accumulation of specialized pathogens at low compared to high species diversity. The corresponding hypotheses are firstly that plants selected in mixtures trade-off reduced defence for increased growth and can evolve stronger mutualistic interactions of plants with soil organisms, in particular arbuscular mycorrhizal fungi (AMF, hypothesis 1). The second hypothesis is that plants selected in monocultures evolved increased defence at the expense of potential growth.

To test hypothesis 1, we conducted a reciprocal inoculation experiment (Klironomos 2002b) of plants selected in monocultures or mixtures with AMF selected in the same monocultures or mixtures. If "home" vs. "away" pairings of plants and AMFs affect plants (and in principle also AMF, but this will not be tested here) differently, this can be an indication for co-adaptation of plants and AMF under the particular conditions of the Jena Experiment.

AMF are soil-borne fungi from the division Glomeromycota, which form symbiotic relationships with most land plants. By penetrating a root parenchyma of the host plant, the fungus extracts plant-derived carbohydrates (Smith & Smith, 2011). In exchange, the fungus provides mineral nutrients to the host (Gianinazzi-Pearson, 1996; van der Heijden *et al.*, 2006). AMF can improve plant survival and growth by increasing nutrient uptake of the host plant (Jones & Smith, 2004; van der Heijden *et al.*, 2006) but also by protecting the plant from the detrimental effects of both above- and belowground pathogens and pests (Newsham *et al.*, 1995; Vannette *et al.*, 2013). Although AMF may promote plant growth, the outcome of the interaction may vary from mutualism to parasitism (Johnson, Graham & Smith 1997; Klironomos 2003; Kiers & Van Der Heijden 2006; Argüello 2013). The dependence of AMF on plant-derived carbon, the short generation time of AMF in comparison to the host plants and its limited dispersal (Vályi *et al.* 2016) provide the potential for rapid adaptation of AMF (Fenchel & Finlay 2004; Rúa *et al.* 2016). Few studies, however, have considered the possibility that AMF may adapt to the host plants in

short-term ecological experiments (Lekberg & Koide, 2014). Comparative field studies which have examined evidence for AMF adaptation without selection imposed by an experimental setting yielded controversial results (Weinbaum, Allen & Allen 1996; Pánková, Raabová & Münzbergová 2014b; Pánková *et al.* 2014a).

Plants host a variety of pathogenic soil-borne micro-organisms with the ability to reduce plant growth or survival by causing root damage or seed and seedling mortality (Bever *et al.*, 2015). Species-specific soil pathogens have been shown to accumulate near the dominant species of the plant community and to consequently inhibit the growth of those species (Mordecai, 2011). Similar accumulations have been found among aboveground pathogens (Rottstock *et al.* 2014). In monocultures, the accumulation of such specialist pathogens and the negative effects on plant growth are thus particularly strong, while in diverse plant communities the detrimental effects of specialist pathogens dilute (van der Putten *et al.* 2013b). Plants may avoid the negative effects of pathogens by investing resources in defences (Bezemer & van Dam, 2005) or by improving interactions with beneficial soil organisms, such as AMF (Newsham *et al.*, 1995). To increase survival, plants in monocultures may thus allocate more resources to defence or may enter more beneficial symbioses. Conversely, in diverse plant communities, interspecific competition rather than pathogen pressure is more likely to drive selection. Consequently, survival in diverse plant communities may rather depend on the ability of the plant to allocate resources to growth instead of defence.

Here we conducted a fully reciprocal inoculation experiment to investigate the specific interactions of plants selected for twelve years in monocultures (monoculture-type plants) and species mixtures (mixture-type plants), and AMF communities co-selected with the studied plants for eight plus three years (monoculture AMF and mixture AMF, respectively, with a mixing of soils after the first eight years of selection). After the co-selection phase we isolated the AMF communities and tested their specific influence on the performance of monoculture- and mixture-type plants. We additionally studied the performance of monoculture-type and mixture-type plants in the absence of AMF (control) and in the presence of external AMF, which did not share a common history with the studied plants. We wanted to study whether long-term selection of plants in monocultures vs. mixtures in a biodiversity experiment (the Jena Experiment) led to co-adaptation of plants and AMF. We expected such co-adaptation would increase the mutualism of plant–AMF associations in home (monoculture-type plants and monoculture AMFs or mixture-type plants and mixture AMFs) compared to away combinations (monoculture-type plants and mixture AMFs or mixture-type plants and monoculture AMFs). In addition, we tested whether monoculture-type plants had been selected for increased defence as a secondary hypothesis.

Materials and Methods

Plant histories

Our study included seven common perennial European grassland species from four different functional groups: one grass (*Festuca rubra* L.), three small herbs (*Plantago lanceolata* L., *Prunella vulgaris* L. and *Veronica chamaedrys* L.), two tall herbs (*Galium mollugo* L. and *Geranium pratense* L.) and one legume (*Lathyrus pratensis* L.). Each of the studied plant species had undergone twelve years of selection from 2002 until 2014 in either plant monocultures (monoculture-type plants) or species mixtures (mixture-type plants) (Fig. 1).

First controlled seed production and soil training

In spring 2010, the entire plant communities of 48 plots (12 monocultures, 12 two-species mixtures, 12 four-species mixtures and 12 eight-species mixtures) of a biodiversity experiment in Jena, Germany, the Jena Experiment (Roscher *et al.* 2004b), were collected as cuttings and transplanted to an experimental garden in Zurich, Switzerland, in identical plant composition for the first controlled sexual reproduction among co-selected plants (Zuppinger-Dingley *et al.* 2014a). Additionally, the top 30 cm soil of the 48 plots was pooled together, mixed and placed back into the excavated locations in the Jena Experiment. In spring 2011, the seedlings produced from the seeds of the first controlled sexual reproduction in Zurich were transplanted back into the mixed soil in the same plots of the Jena Experiment from where the parents had originally been excavated. In these newly established plots, plant communities with identical composition as the original communities were maintained for three years until 2014 to allow them to become re-associated with their own microbial communities.

Second controlled seed production

The seeds used in the present study were obtained from a second controlled sexual reproduction. In March 2014, entire plant communities from the re-established plots in the Jena Experiment were collected and established in their respective communities in plots in the experimental garden in Zurich. For our study, we collected seeds from seven monoculture plots, one four-species mixture plot and six eight-species mixture 1x1 m plots in the experimental garden. The plots were filled with 30 cm of soil (1:1 mixture of garden compost and field soil, pH 7.4, commercial name Gartenhumus, RICOTER Erdaufbereitung AG, Aarberg, Switzerland), and fenced with netting to minimize cross-pollination with plants outside the plots. The seeds of the seven plant species were stored at +4 ˚C for two months. Four weeks before the start of the present experiment, the seeds were surface-sterilized with 7–14 % bleach for 10–45 min to remove any microbiota attached to the seeds and subsequently germinated on 1% water-agar.

Soil collection and inoculum preparation

In March 2014 we collected rhizosphere soil samples attached to the roots of the plants collected in the Jena Experiment (Fig. 1). By then, the soil communities had

undergone three years of community assembly and eight plus three years of potential co-evolution with each of the seven plant species in monocultures (monoculture-type plants) or mixtures (mixture-type plants).

To isolate AMF communities from the sampled rhizosphere soils, we passed deionized water and 25 g of soil sample through a series of sieves and isolated soil particles with a diameter of 32–500 µm using a sugar gradient-centrifugation method (Sieverding 1991). The AMF spores manually collected with a pipet under a microscope at 200-fold magnification. To accumulate the isolated AMF communities, we established trap cultures that consisted of 2 L of 4:1 sand-soil mixture, autoclaved at 120 °C for 99 min, and a monoculture of trap plants of one of each the seven tested plant species (Fig. 1, second row from bottom). All trap cultures received 300–400 AMF spores in 30 ml of deionized water, except for the negative control trap cultures, which received 30 ml of deionized water without AMF spores. We deliberately avoided that the trap plants shared a "community-selection" history (see Chapter one) with the AMF spores collected from the rhizosphere of monoculture- or mixture-type plants of the same species. Therefore we used new seeds from a commercial seed supplier which provided the original seed material for the Jena Experiment (Rieger-Hofmann GmbH, Blaufelden-Raboldshausen, Germany). The seeds were surface-sterilized and pre-germinated on 1% water agar. Each AMF trap culture was replicated twice. After ten months of growth in the glasshouse, we collected a root sample from each trap culture, fixed the root samples in 50 % ethanol, cleared them with 10 % KOH, and stained them with 5 % ink-vinegar (Vierheilig *et al.* 1998). AMF colonization was quantified microscopically. For the trap cultures with fungal colonization, we further quantified the concentration of AMF spores. We isolated AMF spores a 10-g soil sample with the same sieving and centrifugation methods used when setting up the AMF trap-culture pots. The AMF spores we then counted under a microscope. Only five of the seven plant species in the two replicates had sufficient AMF colonization for both monoculture- and mixture-AMF communities. Trap plant cultures that showed fungal root colonization were dried and the plants were harvested at ground level. The roots were harvested and cut into 3–5 cm fragments and the belowground content of the trap cultures was used as soil inoculum in the plant–soil feedback experiment described below.

For the positive control soil treatment we used a trap culture substrate containing *Rhizoglomus irregulare* (Błaszk., Wubet, Renker & Buscot) (Sieverding *et al.* 2015) as the inoculum. We developed the culture for nine months in a substrate of 15 % soil, 65 % sand and 20 % oil binder with *Plantago lanceolata* plants, which had no shared community-selection history with plants or soils from the Jena Experiment. *R. irregulare* (previous names *Glomus intraradices* and *Rhizophagus irregulare*; Sieverding *et al.*, 2015)) is an AMF taxon common in natural grasslands. The *R. irregulare* material we used in the present study was obtained from M.G.A. van der Heijden's Ecological Farming Group of (Agroscope Reckenholz-Tänikon, Zurich, Switzerland).

Plant–soil feedback experiment

To establish the soil treatments of the present study, we filled 1-L pots with gamma-radiated (27–54 kGy) 1:1 (weight/weight) sand-soil mixture and added 9 % (volume/volume) of inoculum without AMF (control), inoculum of AMF isolated from plants grown in monoculture (monoculture AMF) or mixture (mixture AMF), or inoculum containing *Rhizoglomus irregulare.* One monoculture- or mixture-type plant of a single test species was planted in each pot (Fig. 1, lower panel). To standardize the non-AMF microbial community within each pot, we created a microbial wash by filtering 1.2 L of a mixture of unsterilized field soil and the AMF trap culture substrates through a series of sieves and finally through filter paper (MN615, Macherey-Nagel GmbH & Co. KG) with 5 L of deionized water. We confirmed the absence of AMF spores in the filtrate microscopically. Each pot received 10 ml of the microbial-wash filtrate. The experiment included four soil treatments in total, two plant histories (monoculture- and mixture-type plants) and seven plant species in a full factorial design (Table S1). Three species without sufficient AMF colonization in the trap cultures were grown only on the control and *R. irregulare* soil treatments. Combinations of these two soil treatments were replicated five times and the two other AMF treatments were replicated ten times (five times per trap-culture replicate). The 337 pots were randomly arranged within five experimental blocks in a glasshouse compartment with each particular treatment combination and trap-culture replicate occurring only once in each block.

Seed and seedling mortality

Seeds collected from *G. mollugo* mixture-type plants repeatedly developed mould while germinating on the agar plates. As a consequence of the low germination rate of mixture-type *G. mollugo*, the experiment included three *G. mollugo* mixture-type plants less than monoculture-type plants. At the beginning of the experiment the studied plants were infested by fungus gnats (*Bradysia* spp.). This was the cause of some of the plant mortality during the experiment.

Data collection

We cut the plants to 4 cm aboveground three months after planting seedlings into the pots of the different soil treatments (referred to as first harvest). After five months of plant growth, maximum height and average leaf absorbance (SPAD-502Plus Chlorophyll Meter, KONICA MINOLTA, INC., Osaka, Japan) of three representative leaves of each plant were measured and the aboveground biomass was harvested at ground-level (referred to as second harvest). Leaf absorbance of *F. rubra* was not measured because the leaves were too narrow. The biomass of each plant was dried at 70 °C for 48 h and then weighed. We assessed leaf mass per area (LMA) and leaf dry matter content (LDMC) at the second harvest by measuring the area of fresh leaves (LI-3100C Area Meter, LI-COR, Lincoln, USA) immediately after harvest and assessing the weight of the leaves before (fresh weight) and after drying (dry weight). Finally, we estimated the degree of damage on plant aboveground tissues due to powdery mildew (family Erysiphaceae) and two-spotted spider mites (*Tetranychus*

urticae Koch). To determine the AMF colonization of plant roots at the end of the experiment, roots and adhering rhizosphere soil were cut into small fragments and random subsamples of roots were then stored in 50 % ethanol for microscopic quantification of AMF using the same clearing and staining method as described above (Vierheilig *et al.* 1998). All measured traits are listed in Table S2.

Data analyses

We analysed the biomass data, morphological trait measurements, leaf damage estimates and AMF colonization using linear models. Plant survival and AMF colonization was analyzed using analysis of deviance. The results we summarized in analysis of variance (ANOVA) and deviance (ANDEV) tables (McCullagh & Nelder 1998; Schmid *et al.* 2017). The explanatory terms of the models were block, plant functional group, species identity within plant functional group, plant history (monoculture-type vs. mixture-type), soil treatments (four soil treatments or sequence of the following three orthogonal contrasts: control vs. AMF treatments, *R. irregulare* vs. monoculture or mixture AMF and monoculture vs. mixture AMF) and interactions of these. Statistical analyses were conducted using the software product R, version 3.0.2 (R Core Team 2013).

Results

Plant survival

Of the 337 studied plants, 259 plants (77 %) survived at the end of the experiment. Plant survival differed significantly between functional groups and species within functional groups (Table 1, Fig. 2b). Mixture-type plants had on average significantly higher survival than monoculture-type plants with the exception of *G. mollugo* ($P = 0.012$ for the main effect of plant history after exclusion of *G. mollugo*). The lowest observed plant survival occurred in control soil, suggesting that the presence of AMF increased the plants chance of survival. We observed the highest survival in soils containing *R. irregulare* inoculum among the AMF treatments. There were no overall differences between monoculture and mixture AMF and no indication that monoculture-type plants survived better in soil with monoculture AMF or mixture-type plants in soil with mixture AMF. However, there was a significant interaction between plant functional group and monoculture vs. mixture AMF: mixture AMF improved the survival of herb plants, whereas monoculture AMF improved survival of *L. pratensis* plants.

Leaf damage

Mixture-type plants were on average more severely damaged than monoculture-type plants by the pathogens affecting the plants in the glasshouse ($P < 0.001$ for the main effect of plant history; Fig. 2c) This effect was particularly strong in *P. lanceolata* for which mixture-type plants had severe powdery mildew infections (Fig. 2c). Leaf damage also differed significantly between functional groups ($P < 0.001$) and species within functional groups ($P < 0.001$). The interaction of species

with plant history was also significant ($P < 0.001$), which das driven mainly by *P. lanceolata.*

Plant biomass production

Aboveground biomass production differed significantly between plant functional groups and between species within functional groups at both harvests (Table 2, Fig. 3bd). At the first harvest, mixture-type plants of four species, *P. lanceolata*, *P. vulgaris*, *V. chamaedrys* and *G. pratense*, produced more biomass than monoculture-type plants, whereas the opposite was true for the species *F. rubra*, *L. pratensis* and *G. mollugo* (Fig. 3b). The difference in biomass production between monoculture- and mixture-type plants was smaller at the second harvest but still varied significantly among the different plant functional groups (Fig. 3d). Most plant species produced lowest aboveground biomass in control soil and the beneficial effect of AMF was stronger when only those plants for which AMF colonization of roots was detected were included in the three AMF soil treatments (compare Fig. 3 with Fig. S1 and Table 2 with Table S3). Only *V. chamaedrys* mixture-type plants of at the first harvest and *V. chamaedrys of* both monoculture- and mixture-type plants at the second harvest produced more biomass in control soil than in the AMF-inoculated soil treatments (Table 2 and Fig. 3). *Rhizoglomus irregulare* significantly increased biomass production compared with monoculture and mixture AMF at the first harvest but reduced biomass marginally at the second harvest (Table 2, Fig. 3).

AMF colonization

Plant functional group (FG) and species identity explained a significant fraction of the variation in roots with AMF colonization ($P = .016$ for FG, $P < 0.001$ for species identity, Fig. 4b). Monoculture AMF showed greater root colonization than mixture AMF in the species representing legumes (*L. pratensis*) and tall herbs (*G. mollugo*), but mixture AMF led to a larger fraction of roots colonized than monoculture AMF in the species representing small herbs (*P. lanceolata*, *P. vulgaris* and *V. chamaedrys*; $P = 0.009$; Fig. 4b). Inoculation of soil by *R. irregulare* resulted in greater root colonization than monoculture or mixture AMF (Fig. 4) but in this soil treatment colonization was not well correlated with plant biomass production (Fig. 4c). Root colonization was positively correlated with biomass production however for plants growing in soil inoculated with monoculture or mixture AMF (Fig. 4c).

AMF colonization was present in 3/4 of the plants that survived until the end of the experiment in the AMF-inoculated soil treatments (Fig. S2). Plant functional group (FG) and species identity explained a significant fraction of the variation in the presence or absence of AMF colonization ($P < 0.001$ for FG, $P < 0.001$ for species identity, Fig S2). *Veronica chamaedrys* had the lowest AMF colonization, which mirrored its lower biomass production in AMF-inoculated than in control soil. As expected, AMF colonization was absent except for *L. pratensis* in the control soil treatment (Fig. S2), indicating that contamination of pots with AMF spores from outside was unlikely. AMF colonization with *R. irregulare* was more often present for monoculture- than for mixture-type plants and tended to be less present for the

"home" combinations of monoculture-type plants with monoculture AMF and mixture-type plants with mixture AMF than for "away" combinations of monoculture-type plants with mixture AMF and mixture-type plants with monoculture AMF. This was particularly clear for *L. pratensis*, the representative of the legume functional group ($P = 0.039$ for "PH x ST", $P = 0.086$ for "PH x F" and $P = 0.022$ for "FG x PH x F"; Fig. S2).

Influence of soil treatments on plant traits

All measured plant traits differed significantly between plant functional groups and species within functional group (Fig. 5, Tables S5–S8, Figures S3–S5). Monoculture-type plants were generally taller than mixture-type plants, with the exception of the legume *L. pratensis* and the small herb *P. vulgaris* ($P = 0.02$ for "PH" and $P < 0.001$ for "FG x PH" in Table S7; Fig. 5). Mixture-type plants had higher leaf dry matter content (LDMC) than monoculture-type plants of *P. lanceolata*, *P. vulgaris*, *L. pratensis* and *G. pratense,* whereas the opposite was the case for *V. chamaedrys* and *G. mollugo* ($P = 0.037$ in Table S5; Fig. S3). Similarly, mixture-type plants of *P. lanceolata*, *P. vulgaris*, *L. pratensis*, *G. mollugo* and *G. pratense* had higher LMA than monoculture-type plants whereas the opposite was the case for *F. rubra* and *V. chamaedrys* ($P = 0.041$ in Table S6; Fig. S4).

Variation in plant traits was partially explained by plant functional groups ("FG") or species ("SP"), as expected. However, interactions between plant functional groups ("FG") or species ("SP"), plant history ("PH"") and the soil contrast monoculture vs. mixture AMF ("F") also explained some of the variation in plant traits. Interactions with plant history and monoculture vs. mixture AMF (one-degree-of-freedom term) were of specific interest for this study as they test for co-adaptation between plants and AMF grown in monoculture vs. mixture. We wanted to determine whether home-combinations of monoculture-type plants with monoculture AMF and mixture-type plants with mixture AMF differ from away-combinations of monoculture-type plants with mixture AMF and mixture-type plants with monoculture AMF.

The representatives of the legumes (*L. pratensis*) and the tall herbs (*G. mollugo)* functional groups tended to grow taller with mixture than with monoculture AMF, which was not the case for the small herbs ($P < 0.001$ in Table S7, Fig. 5). LMA decreased in treatments with co-selected AMF in mixture-type plants of *L. pratensis* and both plant histories of *V. chamaedrys* ($P < 0.001$ in Table S7, Fig. S4). Co-selected AMF also increased LMA of mixture-type plants of *P. lanceolata* and *G. mollugo*, monoculture-type plants of *L. pratensis* and both plant histories of *P. vulgaris*. Co-selected AMF increased leaf absorbance in the small herbs *P. lanceolata*, *P. vulgaris* and *V. chamaedrys*. In contrast, leaf absorbance was reduced for the legume *L. pratensis* and the tall herb *G. mollugo* ($P = 0.002$ in Table S8, Fig. S5).

Discussion

We hypothesized that monoculture-type plants may have been selected for increased beneficial associations with AMF (hypothesis 1) or improved defence against pathogens (hypothesis 2). We tested our hypotheses with seven plant species belonging to four different functional groups and found mixed evidence for co-adaptation of AMF with plants.

AMF-inoculated soil treatments showed colonization of plant roots, confirming that the inoculation of the soil treatments with AMF spores from the field was successful. However, in approximately a fifth of the plants that had been growing on AMF-inoculated soils, root colonization was not visible at the end of the experiment. The apparent absence of colonization may in part because we estimated AMF colonization from a random sub-sample of roots rather than of the entire root system. As we could not identify which plants were false negatives for AMF colonization, we conducted separate analyses either including all plants of the present study or only including plants with visual AMF colonization.

The presence/absence of colonization and the proportion of roots colonized with AMF varied between plant functional groups and species. We found no indication, however, that monoculture AMF associated more intensively with monoculture-type plants and mixture AMF with mixture-type plants. In contrast, monoculture AMF tended to have greater colonization than mixture AMF in legumes and tall herbs but not in small herbs. Furthermore, we did not observe any difference in the survival of monoculture- or mixture-type plants in response to monoculture or mixture AMF. Rather both monoculture and mixture AMF similarly improved biomass production of monoculture- and mixture-type plants.

With respect to plant traits, however, we did found some evidence of co-adaptation between monoculture-type plants and monoculture AMF and between mixture-type plants and mixture AMF (home combinations). Namely, co-selected AMF increased leaf absorbance and LMA in three tall and two small herb species. The opposite response was observed for leaf absorbance in representatives of legumes and tall herbs and for LMA in one small herb species. Increased leaf absorbance and high LMA are related to higher area-based nitrogen content (Niinemets 1997; Moran *et al.* 2000) suggesting that co-selected AMF may have improved the nitrogen uptake of two of the small herbs. In contrast, the small herb *V. chamaedrys* performed poorly when grown in the presence of AMF. A previous study with two species of *Prunella* found strong effects of co-occurring AMF on plant aboveground morphological traits (Streitwolf-Engel *et al.*, 1997). We found similar effects for the interactions of AMF, which differed in their selection history in plant monoculture vs. mixture, with several of the tested plant species.

Our results did not in support our hypothesis that co-selection of plants and AMF in plant monocultures or mixtures leads to more beneficial associations between plants and AMF. They rather suggest co-adaptation with AMF may vary between plant functional groups and species, leading to beneficial, neutral or even detrimental effects for the AMF colonized plant. Previous studies examining the co-

adaptation of AMF and plants have found variable results ranging from those supporting co-adaption (Weinbaum *et al.*, 1996; Pánková *et al.*, 2014a) to those that do not (Pánková *et al.*, 2014b). A recent meta-analysis suggested the variability in the outcomes of such studies is influenced by the origin of the soil in which the co-adaptation is tested, because co-adaption was more commonly found when plant, soil and AMF shared a common origin (Rúa *et al.*, 2016). The present study, however, showed that eight plus three years of co-selection of plants and AMF did not generally result in more beneficial associations. It is conceivable that other factors may have resulted in the beneficial effects of a common plant and soil history in previous studies (Rúa *et al.*, 2016, Zuppinger-Dingley *et al*. 2016).

We found significant differences between the AMF collected from the Jena Experiment and the "control" AMF *R. irregulare*, which did not share a common selection history with the experimental plants. Interestingly, *R. irregulare* colonization was initially greater than both mixture- or monoculture-AMF and plant biomass was higher for plants inoculated with this AMF species. At the second harvest, however, the effect of *R. irregulare* colonization on plant biomass was marginally negative. Increasing AMF diversity may stabilize the outcome of plant–AMF symbiosis (van der Heijden *et al.* 1998). The more positive effect of AMF colonization on final plant biomass with monoculture and mixture AMF, than with *R. irregulare*, might therefore in part have been a result of AMF diversity effects. The inoculum with *R. irregulare* represented a single AMF species whereas monoculture and mixture AMF inocula likely included several AMF species. Because competition between AMF species tends to reduce the overall success of AMF colonization (Engelmoer, Behm & Toby Kiers 2014), the greater colonization in the present study may be due to the absence of AMF competitors in the *R. irregulare* soil inoculum.

The accumulation of specialized pathogens is a well-known phenomenon in monocultures, which may drive differential selection of plants at low vs. high species diversity (Zuppinger-Dingley *et al.* 2016a). We hypothesized that monoculture-type plants may have been selected for improved pathogen defence at the cost of reduced growth potential (hypothesis 2). Because specialized pathogens tend to dilute in diverse plant communities (Eisenhauer, Reich & Scheu 2012), we expected monoculture- type plants and not mixture-type plants to trade-off growth potential to increase defence against pathogens. The increase in biomass production of mixture-type plants in comparison to monoculture-type plants for four of our seven plant species supported this hypothesis. But for the three other study species monoculture-type plants produced more biomass than mixture-type plants. To find effects, we had to look at aboveground pathogen damage. Mixture-type plants had greater leaf damage caused by the fungal pathogen powdery mildew, generally confirming the hypothesis that monoculture-type plants evolved increased defence. We observed particularly severe infections by powdery mildew in mixture-type plants of *P. lanceolata*, suggesting, in agreement with Engelmoer et al. (2014), that monoculture-

type plants of *P. lanceolata* may have been subjected to particularly strong selection pressure for pathogen defence in comparison with mixture-type plants.

Conclusions

We found limited evidence for co-adaptation of plants and AMF after eight plus three years of co-selection in plant monocultures vs. mixtures. Furthermore, in those cases for which we did find co-adaptation it was often detrimental to the plant. Our results did not support the hypothesis that monoculture-or mixture-type plants may be selected for more beneficial mutualism with their home AMF, i.e. monoculture or mixture AMF, respectively. This suggests that co-adaptation between plants and AMF in plant biodiversity experiments does not follow a general pattern leading to increased mutualism but rather depends on the specificity of the context and more resembles an arms race in which sometimes the outcome may be reduced mutualism, depending on the plant functional group or species involved. However, we did find consistent evidence that surviving monoculture-type plants may have been selected for improved defence, potentially in response to an accumulation of specialized pathogens in monocultures over time. Here we examined the potential co-adaptation of AMF and monoculture- vs. mixture-type plants to disentangle the mechanisms underlying the previously observed evolution of positive plant–soil feedbacks among monoculture-type plants in contrast to mixture-type plants in biodiversity experiments (Zuppinger-Dingley *et al.* 2016a). From the present study, we conclude that other beneficial soil organisms or increased defences against monoculture- but not mixture-specific pathogens may underlie these previously observed effects. Finally, the lower defence potential of mixture- in comparison with monoculture-type plants offers an explanation for the previously reported negative plant–soil feedbacks in mixture-type plants (Zuppinger-Dingley *et al.* 2016a).

Acknowledgements

We thank M. Furler, D. Topalovic, D. Trujillo Villegas and T. Zwimpfer for technical assistance, D. Flynn, V. Yadav and D. Zuppinger-Dingley for the establishment of the field plots and A. Ferrari, S. Karbin, J. Moser and Y. Xu for help with measurements. This study was supported by the Swiss National Science Foundation (grants number 147092 and 166457 to B. S.) and the University Research Priority Program Global Change and Biodiversity of the University of Zurich. The Jena Experiment is supported by the German Science Foundation (FOR 1451, SCHM 1628/5-2).

Author contributions

T.H., C.W., S.J.V.M., D.Z.D and B.S. conceived the study, T.H. carried out the experiment and T.H., C.W., M.W.S. and B.S. analyzed the data. T.H., C.W., S.J.V.M and B.S. wrote the manuscript with the other authors contributing to revisions.

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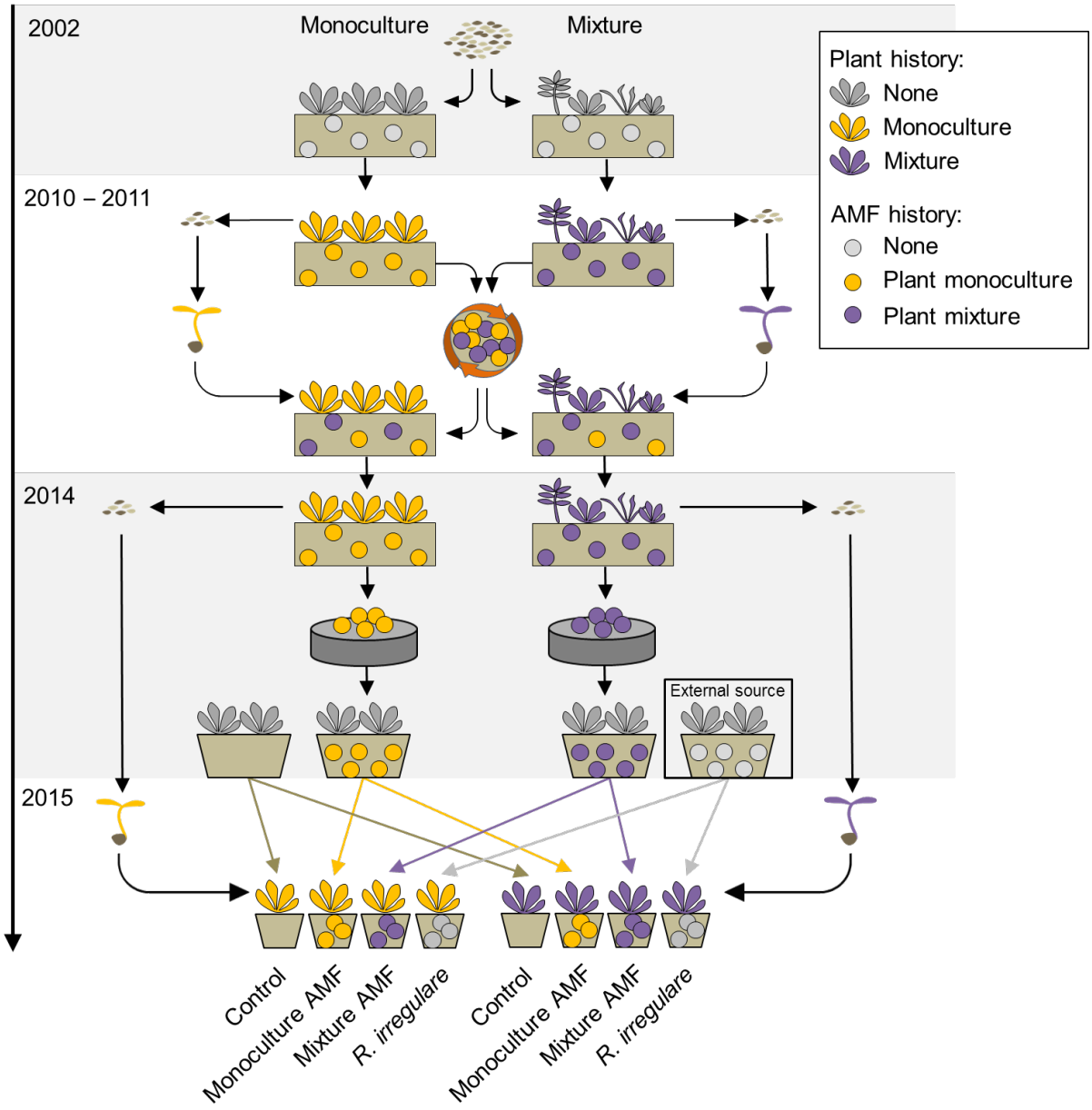

Fig. 1 Experimental design. Plant monocultures and mixtures in the Jena Experiment were sown in 2002 and maintained until 2010. In 2010, the plants of 48 plots underwent a first controlled seed production event and the soil of the plots was pooled, mixed and placed back to the excavated locations. In spring 2011, the seedlings produced were transplanted back to the mixed soil in the same plots from which their parents were excavated. The plant communities could then again associate with their own microbial communities potentially co-assembling and co-evolving until 2014. In spring 2014, the plants underwent a second controlled seed production event, and the AMF spores from their rhizosphere soil were isolated. The isolated AMF communities accumulated in trap-cultures for ten months with trap plants lacking a common selection history with the AMF spores. Control trap-cultures without AMF spores were established as negative control. Four soil treatments were

used: 1) pots with sterile soil and 9 % inoculum without AMF, 2) inoculum of AMF isolated from plants grown in monoculture, 3) inoculum of AMF isolated from plants grown in mixture and 4) inoculum containing *Rhizoglomus irregulare*. Finally, the plants with a selection history in either monoculture (monoculture-type plants) or mixture (mixture-type plants) were planted individually into the prepared pots.

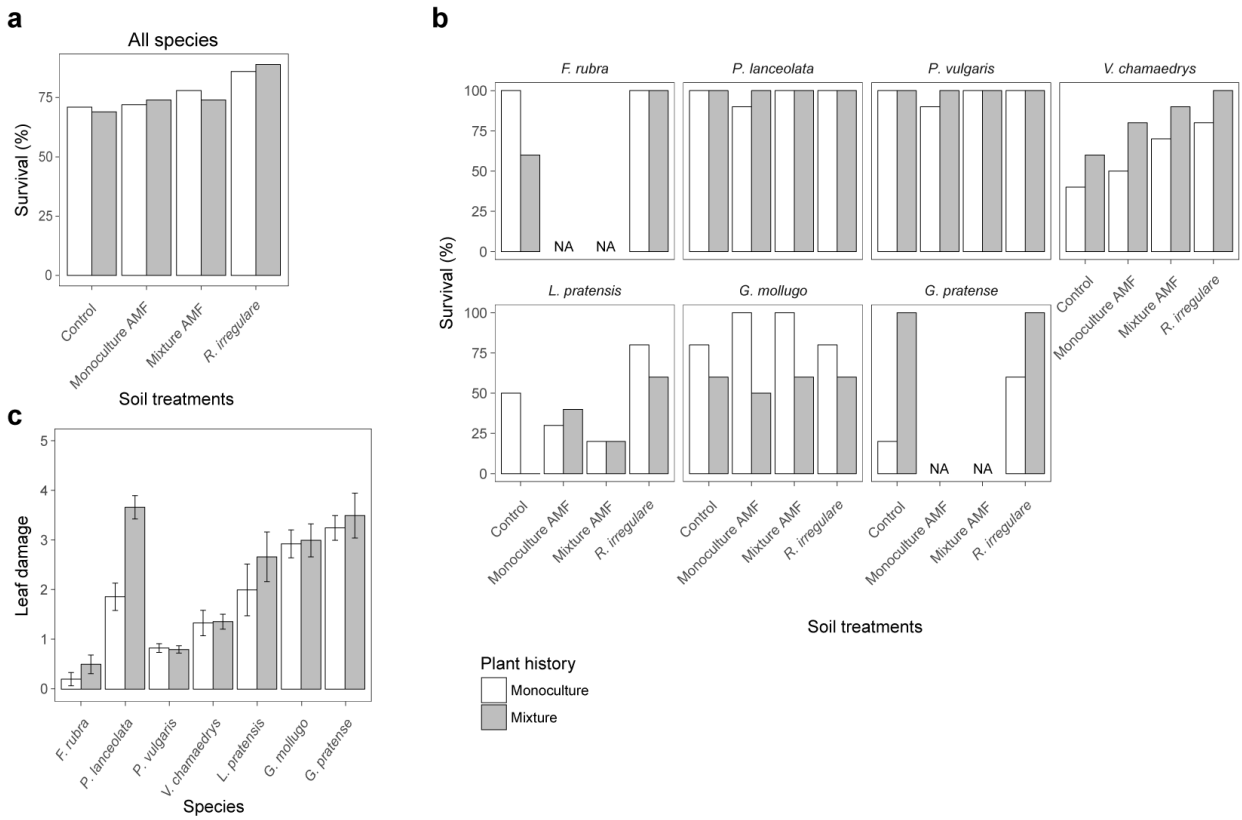

Fig. 2 Survival and leaf damage of monoculture- (white bars) and mixture-type plants (grey bars) in the four soil treatments. **a**, across all species; **b**, by species. Bars are the proportion al percentage of survivors of all planted individuals of that species within selection history in the experiment. **c,** Amount of leaf damage estimated from no damage (0) to strong damage (5) of monoculture-type plants and mixture-type plants. Bars represent means ± standard errors.

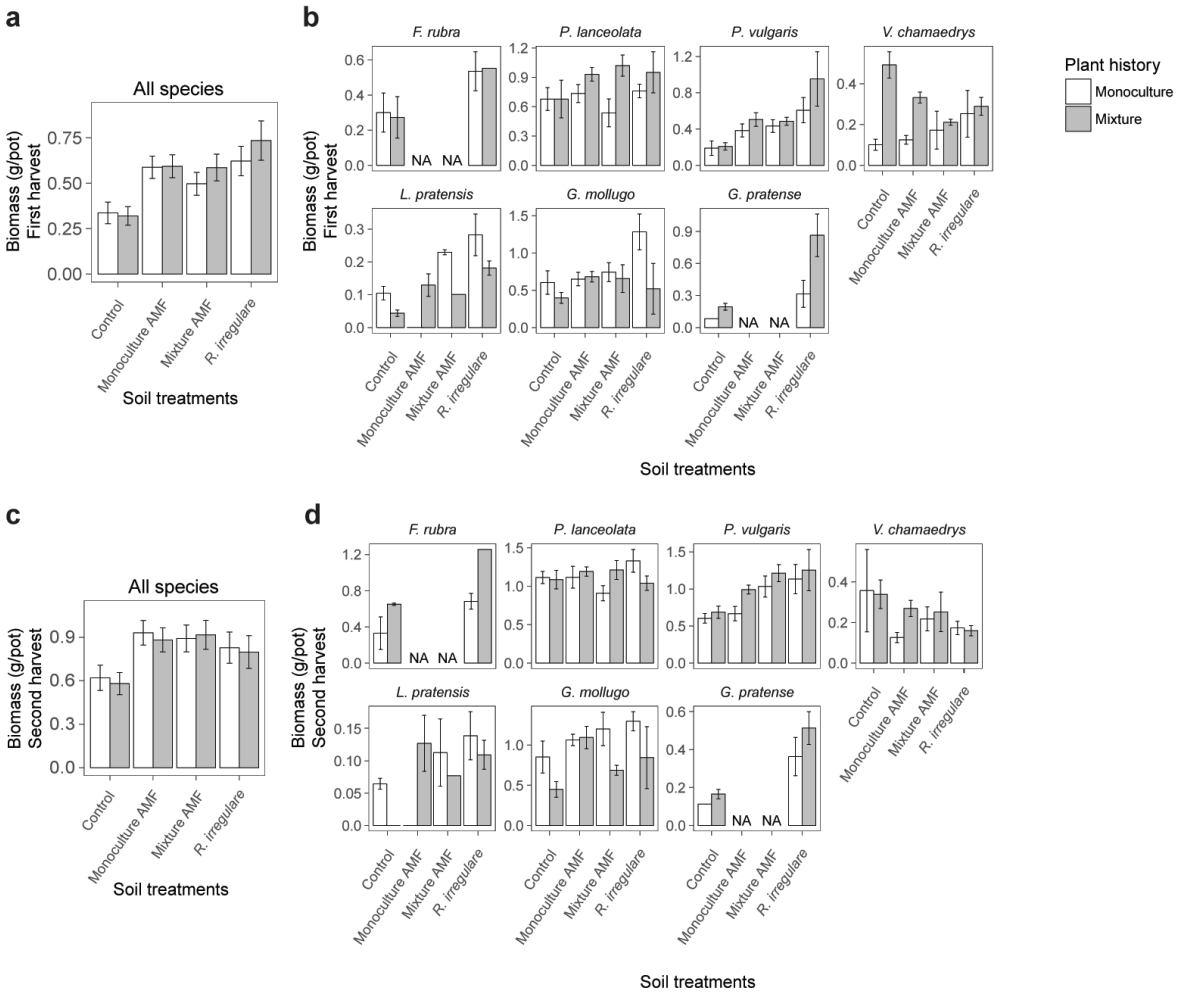

Fig. 3 Plant aboveground biomass production of monoculture-type plants (white bars) and mixture-type plants (grey bars) on each of the four soil treatments. Plants for which AMF colonization failed were excluded (except for soil treatment "Control"). **a**, at the first harvest across all species. **b**, at the first harvest for each species individually. **c,** same as (a), but at the second harvest. **d,** same as (b) but at the second harvest. Bars represent means ± standard errors.

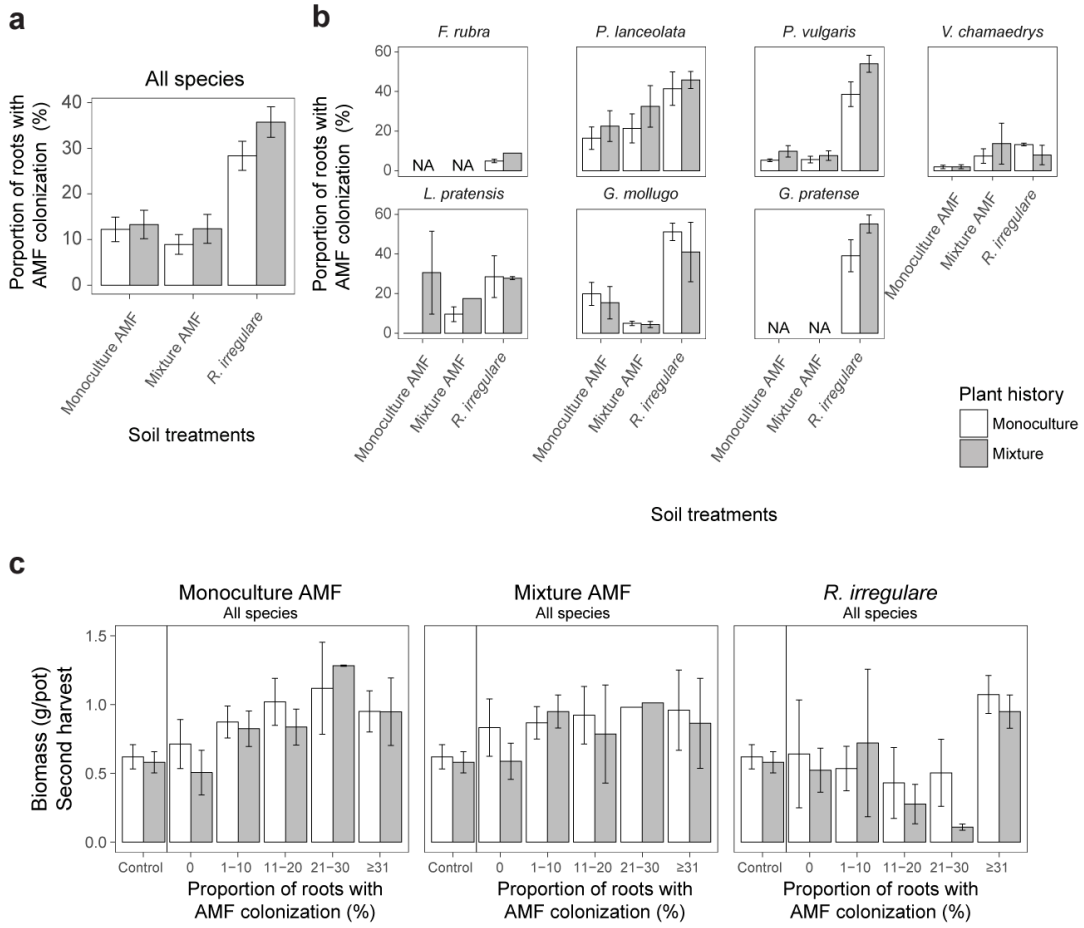

Fig. 4 Percentage of AMF colonization in the roots of monoculture-type plants (white bars) and mixture-type plants (grey bars). **a**, across all species; **b**, by species. Only plants with successful AMF-colonization (i.e. colonization > 0 %) were included in calculations and preparation of this figure. Bars are means ± standard errors. "NA" indicates that no plants were available of that species for the particular soil treatment. **c,** aboveground biomass production of monoculture- and mixture-type plants in control soil in dependence of different levels of monoculture AMF colonization (left panel), mixture AMF colonization (middle panel) and *R. irregulare* colonization (right panel). Data are across species. Bars represent means ± standard errors.

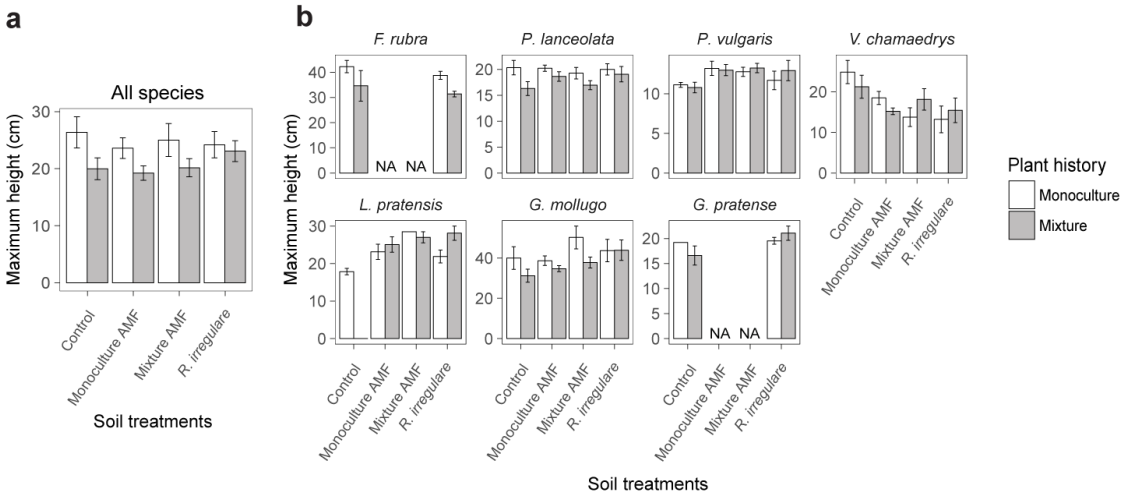

Fig. 5. Maximum height of monoculture-type plants (white bars) and mixture-type plants (grey bars) in the four soil treatments. **a**, across all species including all surviving plants; **b**, by species including all surviving plants. Bars represent means ± standard errors. "NA" indicates that no plants were available of that species for the particular soil treatment.

Table 1. Analysis of deviance (ANDEV) for plant survival.

Source of variation	Df	%-DV	*P*
Block	4	0.7	0.436
Functional group (FG)	3	18.5	**<0.001**
Species within FG (SP)	3	8.0	**<0.001**
Plant history (PH)	1	0.0	0.633
Soil treatment (ST)	3	2.6	**0.002**
Control vs. AMF treatments (C)	1	1.3	**0.008**
R. irregulare vs. monoculture or mixture AMF (R)	1	1.1	**0.015**
Monoculture vs. mixture AMF (F)	1	0.2	0.253
FG × PH	3	2.7	**0.003**
FG × ST	7	3.0	**0.018**
FG × C	3	0.4	0.593
FG × R	2	1.5	**0.017**
FG × F	2	1.2	**0.043**
SP × PH	3	5.1	**<0.001**
Residuals	308	53.7	

Notes: Df, degrees of freedom; %-DV, proportion of total deviance; *P*, error probability

Table 2. Analysis of variance (ANOVA) for plant biomass at the first harvest (a) and at the second harvest (b). Significant and marginally significant effects (P < 0.1) are indicated in bold.

a) Source of variation	Df	%-SS	*P*
Block	4	7.8	**<0.001**
Functional group (FG)	3	12.8	**<0.001**
Species within FG (SP)	3	15.2	**<0.001**
Plant history (PH)	1	0.5	0.111
Soil treatment (ST)	3	7.8	**<0.001**
Control vs. AMF treatments (C)	1	4.0	**<0.001**
R. irregulare vs. monoculture or mixture AMF (R)	1	3.7	**<0.001**
Monoculture vs. mixture AMF (F)	1	0.0	0.750
FG × PH	3	2.6	**0.007**
FG × ST	7	1.0	0.660
FG × C	3	0.4	0.562
FG × R	2	0.4	0.420
FG × F	2	0.3	0.549
SP × PH	3	2.3	**0.013**
SP × ST	7	2.5	0.104
SP × C	3	1.8	**0.040**
SP × R	2	0.7	0.182
SP × F	2	0.0	0.898
Residuals	173	36.1	

b) Source of variation	Df	%-SS	*P*
Block	4	7.5	**<0.001**
Functional group (FG)	3	19.6	**<0.001**
Species within FG (SP)	3	32.2	**<0.001**
Plant history (PH)	1	0.0	0.794
Soil treatment (ST)	3	3.6	**<0.001**
Control vs. AMF treatments (C)	1	3.0	**<0.001**
R. irregulare vs. monoculture or mixture AMF (R)	1	0.6	0.053
Monoculture vs. mixture AMF (F)	1	0.0	0.738
FG × PH	3	2.7	**0.001**
FG × ST	7	0.3	0.972
FG × C	3	0.2	0.792
FG × R	2	0.1	0.849
FG × F	2	0.1	0.831
SP × PH	3	0.6	0.229
SP × ST	7	2.9	**0.008**
SP × C	3	2.1	**0.003**
SP × R	2	0.1	0.703
SP × F	2	0.7	0.098
Residuals	163	23.9	

Notes: Df, degrees of freedom; %-SS, proportion of total sum of squares; *P*, error probability. Plants where AMF colonization failed excluded (except for soil treatment "Control").

Supporting Information

Assessment of soil N and P content at the beginning of the experiment

We conducted Olsen-P and N-mineralization analyses to confirm that the content of phosphate and ammonium, respectively, did not vary in the inoculated substrate at the beginning of the experiment. For Olsen-P analysis, phosphorus was extracted from a 2 g soil sample following the procedure of Olsen et al. (1954). We incubated 20 g of soil sample at 40 °C for 7 days in waterlogged conditions for N-mineralization (Keeney 1982) and then extracted ammonium with 2M KCl (Kandeler & Gerber 1988). The extracted phosphorus and nitrogen content was measured using The San^{++} Continuous Flow Analyzer (Skalar Analytical B.V., Breda, The Netherlands). The inoculated experimental substrate had a phosphate content of 3.76 mg kg^{-1} and an ammonium content of 4.58 mg kg^{-1} which did not vary among the inoculum treatments (soil P: $F_{4, 17} = 1.53$, P = 0.238 and soil N: $F_{4, 17} = 1.53$, P = 0.239).

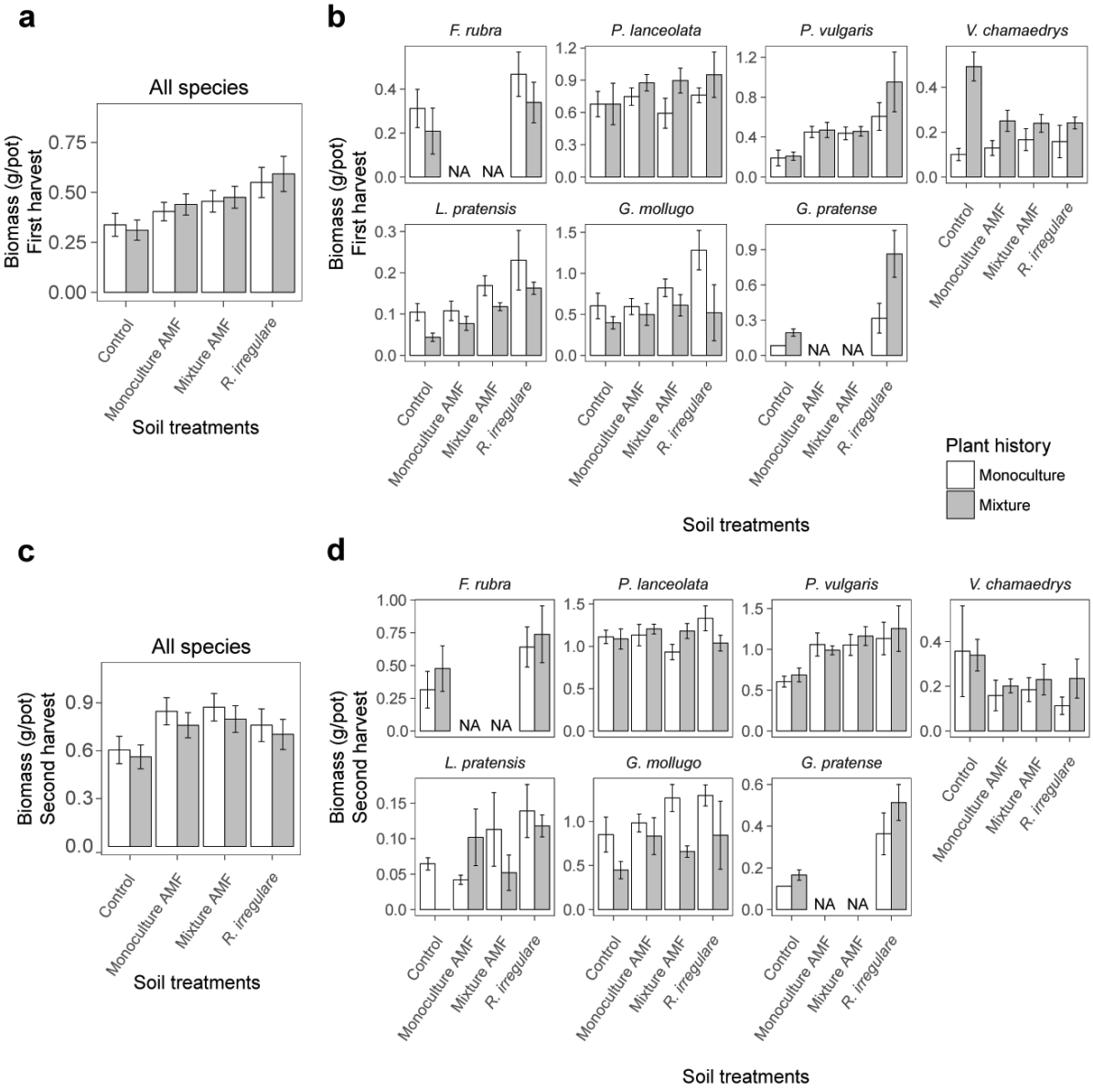

Fig. S1 Plant aboveground biomass production of monoculture- (white bars) and mixture-type plants (grey bars) on each of the four soil treatments for all surviving plants. **a**, at the first harvest across all species. **b**, at the first harvest by species. **c,** same as (a), but at the second harvest. **d,** same as (b) but at the second harvest. Bars represent means ± standard errors.

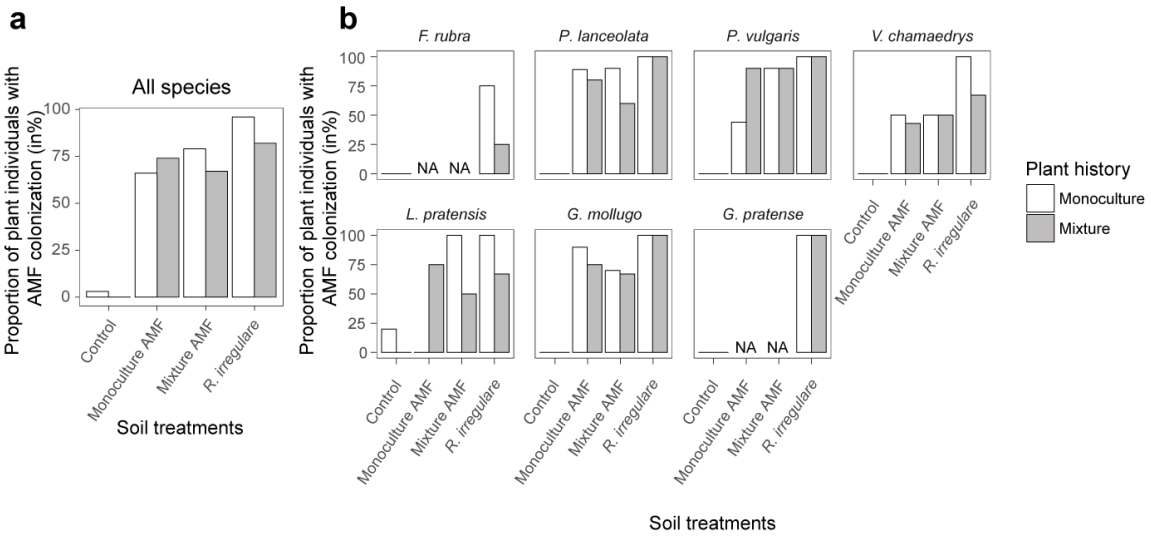

Fig. S2. Proportion of plant individuals with AMF colonization in the roots of monoculture- (white bars) and mixture-type plants (grey bars): **a**, across all species; **b**, by species. The bars are proportions of colonized plants out of all surviving experimental plants. "NA" indicates that no plants were available of that species for the particular soil treatment.

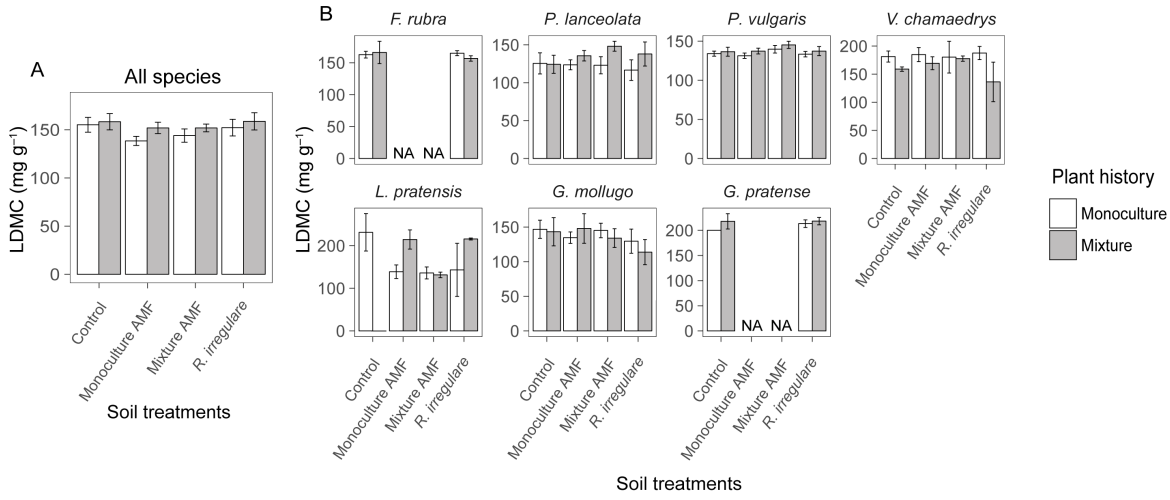

Figure S3. LDMC of monoculture- (white bars) and mixture-type plants (grey bars) in the four soil treatments: **a**, across all species including all surviving plants; **b**, by species including all surviving plants. Bars represent means ± standard errors. "NA" indicates that no plants were available of that species for the particular soil treatment.

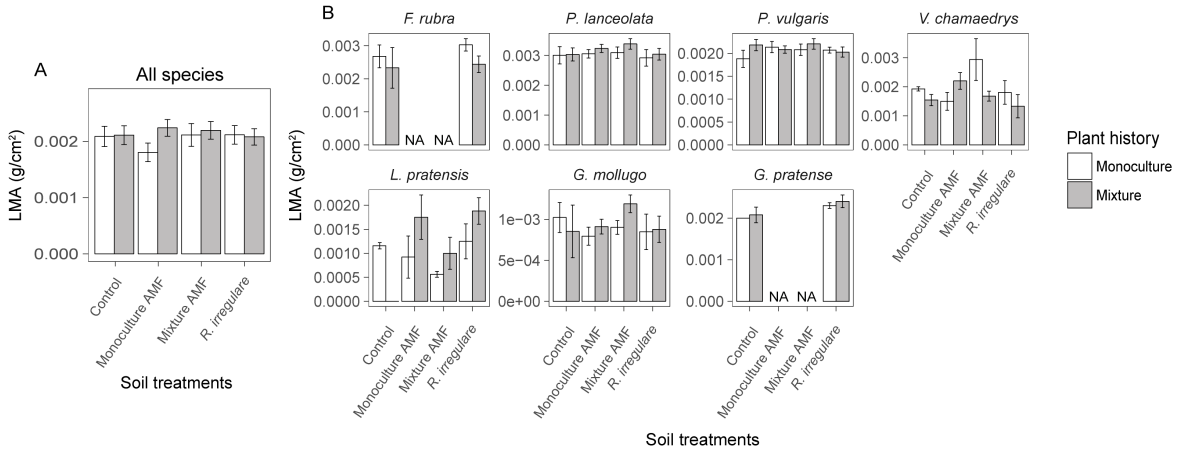

Figure S4. LMA of monoculture- (white bars) and mixture-type plants (grey bars) in the four soil treatments: **a**, across all species including all surviving plants; **b**, by species including all surviving plants. Bars represent means ± standard errors. "NA" indicates that no plants were available of that species for the particular soil treatment.

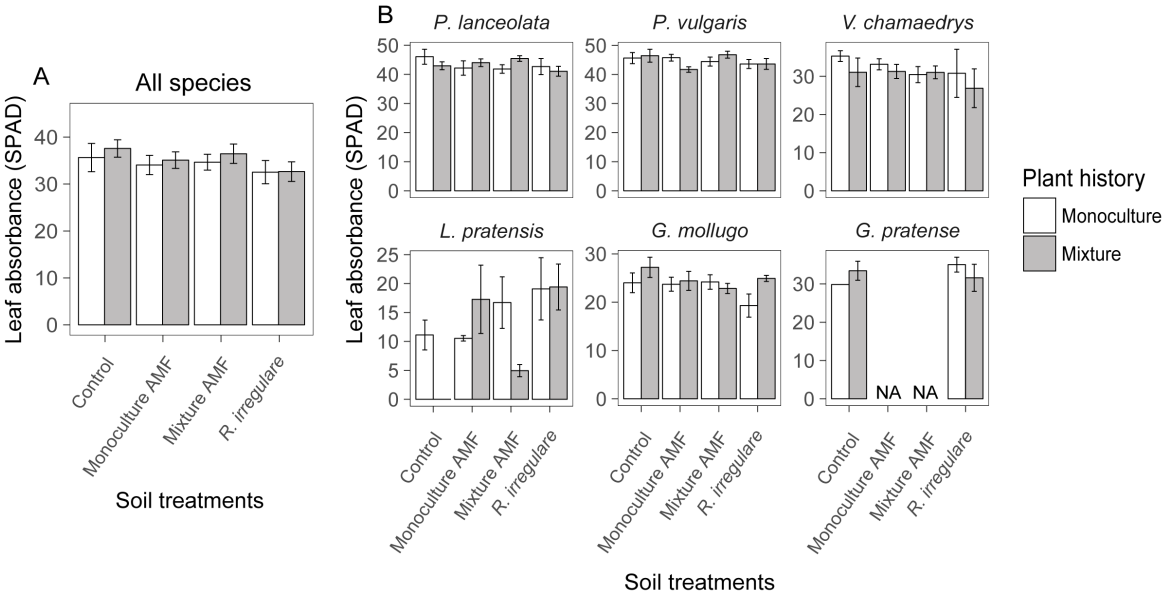

Figure S5. Leaf absorbance (SPAD) of monoculture- (white bars) and mixture-type plants (grey bars) on the four soil treatments: **a**, across all species including surviving plants; **b**, by species including all surviving plants. Bars represent means ± standard errors. "NA" indicates that no plants were available of that species for the particular soil treatment.

Table S1 Experimental design. Number of replicates for monoculture- and mixture-type plants of four functional groups and seven species grown on the four soil treatments of the experiment.

		Soil treatments			
Species	Plant history	Control	Monoculture AMF	Mixture AMF	*R. irregulare*
Festuca rubra	Monoculture	5	0	0	5
	Mixture	5	0	0	5
Plantago lanceolata	Monoculture	5	10	10	5
	Mixture	5	10	10	5
Prunella vulgaris	Monoculture	5	10	10	5
	Mixture	5	10	10	5
Veronica chamaedrys	Monoculture	5	10	10	5
	Mixture	5	10	10	5
Lathyrus pratensis	Monoculture	5	10	10	5
	Mixture	5	10	10	5
Galium mollugo	Monoculture	5	10	10	5
	Mixture	5	9	8	5
Geranium pratense	Monoculture	5	0	0	5
	Mixture	5	0	0	5
		N = 337			

Table S2. Plant traits measured at the experimental plant age.

Measured plant trait	Unit	Plant age (weeks)
Aboveground biomass, first harvest	g dry weight/pot	12
Aboveground biomass, second harvest	g dry weight/pot	20
Leaf damage	severity 0–5 (none to high)	20
AMF colonization	presence/absence	20
Proportion of roots with AMF colonization	%	20
Leaf absorbance	SPAD (0-50)	19
Leaf dry matter content (LDMC)	mg dry weight g^{-1} fresh weight	20
Leaf mass per area (LMA)	g dry weight/cm^2	20
Maximum height	cm	19

Note: SPAD values are index values, defined by the manufacturer of the chlorophyll content measuring device, that indicate the relative amount of chlorophyll present in the leaf.

Table S3. Analysis of variance (ANOVA) for plant biomass at the first harvest (a) and at the second harvest (b). Significant and marginally significant effects (P < 0.1) are indicated in bold.

a) Source of variation	Df	%-SS	*P*
Block	4	6.9	**<0.001**
Functional group (FG)	3	18.0	**<0.001**
Species within FG (SP)	3	20.2	**<0.001**
Plant history (PH)	1	0.1	0.315
Soil treatment (ST)	3	4.5	**<0.001**
Control vs. AMF treatments (C)	1	2.2	**<0.001**
R. irregulare vs. monoculture or mixture AMF (R)	1	2.2	**<0.001**
Monoculture vs. mixture AMF (F)	1	0.1	0.340
FG × PH	3	2.1	**0.001**
FG × ST	7	1.5	0.119
FG × C	3	0.4	0.371
FG × R	2	0.5	0.121
FG × F	2	0.5	0.124
SP × PH	3	1.9	**0.002**
SP × ST	7	2.7	**0.005**
SP × C	3	1.6	**0.006**
SP × R	2	1.0	**0.022**
SP × F	2	0.1	0.715
Residuals	266	33.7	

b) Source of variation	Df	%-SS	*P*
Block	4	6.8	**<0.001**
Functional group (FG)	3	16.5	**<0.001**
Species within FG (SP)	3	41.1	**<0.001**
Plant history (PH)	1	0.1	0.435
Soil treatment (ST)	3	2.8	**<0.001**
Control vs. AMF treatments (C)	1	2.4	**<0.001**
R. irregulare vs. monoculture or mixture AMF (R)	1	0.3	0.074
Monoculture vs. mixture AMF (F)	1	0.0	0.813
FG × PH	3	1.9	**0.001**
FG × ST	7	0.3	0.883
FG × C	3	0.1	0.730
FG × R	2	0.0	0.954
FG × F	2	0.2	0.448
SP × PH	3	0.4	0.235
SP × ST	7	2.2	**0.004**
SP × C	3	1.9	**<0.001**
SP × R	2	0.1	0.714
SP × F	2	0.2	0.316
Residuals	223	22.8	

Notes: Df, degrees of freedom; %-SS, proportion of total sum of squares; *P*, error probability. All surviving plants were included in this analysis.

Table S4. ANDEV for proportion of plant individuals with AMF colonization in the roots.

Source of variation	Df	%-DV	*P*
Block	4	0.2	0.827
Functional group (FG)	3	2.4	**<0.001**
Species within FG (SP)	3	2.4	**<0.001**
Plant history (PH)	1	0.1	0.502
Soil treatment (ST)	3	28.9	**<0.001**
Control vs. AMF treatments (C)	1	26.4	**<0.001**
R. irregulare vs. monoculture or mixture AMF (R)	1	2.5	**<0.001**
Monoculture vs. mixture AMF (F)	1	0.0	0.619
FG × PH	3	0.4	0.415
FG × ST	7	0.6	0.705
FG × C	3	0.0	1.000
FG × R	2	0.1	0.684
FG × F	2	0.5	0.129
SP × PH	3	1.0	**0.049**
SP × ST	7	0.8	0.549
SP × C	3	0.0	1.000
SP × R	2	0.2	0.403
SP × F	2	0.6	0.110
PH × ST	3	1.1	**0.039**
PH × C	1	0.0	1.000
PH × R	1	0.7	**0.015**
PH × F	1	0.4	0.086
FG × PH × ST	7	1.0	0.395
FG × PH × C	3	0.0	1.000
FG × PH× R	2	0.0	1.000
FG × PH× F	2	1.0	**0.022**
Residuals	223	28.9	

Notes: Df, degrees of freedom; %-DV, proportion of total deviance; *P*, error probability

Table S5. ANOVA for plant LDMC.

Source of variation	Df	%-SS	*P*
Block	4	2.5	**0.024**
Functional group (FG)	3	5.5	**<0.001**
Species within FG (SP)	3	27.3	**<0.001**
Plant history (PH)	1	0.2	0.346
Soil treatment (ST)	3	0.4	0.574
Control vs. AMF treatments (C)	1	0.0	0.732
R. irregulare vs. monoculture or mixture AMF (R)	1	0.4	0.210
Monoculture vs. mixture AMF (F)	1	0.1	0.588
FG × PH	3	0.9	0.241
FG × ST	7	5.3	**0.002**
FG × C	3	3.1	**0.004**
FG × R	2	0.8	0.182
FG × F	2	1.4	**0.041**
SP × PH	3	1.9	**0.037**
Residuals	227	50.3	

Notes: Df, degrees of freedom; %-SS, proportion of total sum of squares; *P*, error probability.

Table S6. ANOVA for plant LMA.

Source of variation	Df	%-SS	*P*
Block	4	3.8	**<0.001**
Functional group (FG)	3	30.4	**<0.001**
Species within FG (SP)	3	26.0	**<0.001**
Plant history (PH)	1	0.0	0.920
Soil treatment (ST)	3	0.6	0.201
Control vs. AMF treatments (C)	1	0.3	0.149
R. irregulare vs. monoculture or mixture AMF (R)	1	0.2	0.244
Monoculture vs. mixture AMF (F)	1	0.2	0.273
FG × PH	3	0.9	0.081
FG × ST	7	1.4	0.150
FG × C	3	0.1	0.777
FG × R	2	0.8	**0.045**
FG × F	2	0.5	0.175
SP × PH	3	1.1	**0.041**
SP × ST	7	0.7	0.581
SP × C	3	0.2	0.629
SP × R	2	0.4	0.237
SP × F	2	0.1	0.600
PH × ST	3	0.4	0.441
PH × C	1	0.0	0.954
PH × R	1	0.0	0.653
PH × F	1	0.3	0.115
FG × PH × ST	6	0.3	0.879
FG × PH × C	2	0.1	0.695
FG × PH × R	2	0.0	0.896
FG × PH × F	2	0.2	0.488
SP × PH × ST	7	2.6	**0.007**
SP × PH × C	3	0.1	0.848
SP × PH × R	2	0.0	0.988
SP × PH × F	2	2.4	**<0.001**
Residuals	204	26.1	

Notes: Df, degrees of freedom; %-SS, proportion of total sum of squares; *P*, error probability.

Table S7. ANOVA for maximum plant height.

Source of variation	Df	%-SS	*P*
Block	4	0.8	0.065
Functional group (FG)	3	54.9	**<0.001**
Species within FG (SP)	3	16.7	**<0.001**
Plant history (PH)	1	0.5	**0.020**
Soil treatment (ST)	3	0.3	0.323
Control vs. AMF treatments (C)	1	0.1	0.345
R. irregulare vs. monoculture or mixture AMF (R)	1	0.0	0.661
Monoculture vs. mixture AMF (F)	1	0.2	0.122
FG × PH	3	1.6	**0.001**
FG × ST	7	2.1	**0.001**
FG × C	3	0.7	**0.043**
FG × R	2	0.1	0.692
FG × F	2	1.3	**0.001**
SP × PH	3	0.6	0.071
Residuals	230	20.1	

Notes: Df, degrees of freedom; %-SS, proportion of total sum of squares; *P*, error probability.

Table S8. ANOVA for plant leaf absorbance.

Source of variation	Df	%-SS	*P*
Block	4	3.2	**<0.001**
Functional group (FG)	2	54.0	**<0.001**
Species within FG (SP)	3	20.2	**<0.001**
Plant history (PH)	1	0.0	0.983
Soil treatment (ST)	3	0.2	0.547
Control vs. AMF treatments (C)	1	0.1	0.196
R. irregulare vs. monoculture or mixture AMF (R)	1	0.0	0.525
Monoculture vs. mixture AMF (F)	1	0.0	0.831
FG × PH	2	0.1	0.751
FG × ST	6	1.2	**0.031**
FG × C	2	0.3	0.203
FG × R	2	0.7	**0.015**
FG × F	2	0.2	0.298
SP × PH	3	0.4	0.224
SP × ST	7	0.2	0.900
SP × C	3	0.1	0.753
SP × R	2	0.0	0.881
SP × F	2	0.1	0.507
PH × ST	3	0.0	0.935
PH × C	1	0.0	0.966
PH × R	1	0.0	0.656
PH × F	1	0.0	0.638
FG × PH × ST	5	1.3	**0.008**
FG × PH × C	1	0.1	0.270
FG × PH × R	2	0.2	0.385
FG × PH × F	2	1.1	**0.002**
Residuals	200	16.4	

Notes: Df, degrees of freedom; %-SS, proportion of total sum of squares; *P*, error probability.

DISCUSSION

Nature has introduced great variety into the landscape, but man has displayed a passion for simplifying it. Thus, he undoes the built-in checks and balances by which nature holds the species within bounds.

- Rachel Carson in Silent Spring (1962)

This dissertation aimed to determine the role of community evolution for ecosystem functioning and to infer information about the speed of such evolutionary processes in grasslands. For this purpose, I conducted experiments in the glasshouse and in the field. In a large biodiversity experiment I investigated ecosystem functioning (Chapter 1) and ecosystem stability (Chapter 1). In two glasshouse pot experiments, I studied biodiversity effects (Chapter 3), epigenetic and genetic variation (Chapter 4) and the role of plant–soil feedbacks by testing for co-adaptation between arbuscular mycorrhizal fungi (AMF) and plants (Chapter 5). This holistic approach at the intersection between ecology and evolutionary biology allowed me to draw important conclusions and to consequently advance the knowledge in the field of community ecology.

Before I start discussing and contextualizing the findings of the present dissertation, I would like to emphasize the importance of biodiversity research for our society and place my research in a broader and more applied context.

The importance of biodiversity–ecosystem functioning (BEF) research for humanity

Biodiversity buffers the impact of climate change, increases agricultural food production and increases or enables the provisioning of other goods such as timber or fish (Cardinale *et al.* 2012). It is obvious that these benefits, so-called ecosystem services, are crucial for human wellbeing and that our society should preserve global and local genetic and species diversity as far as possible. However, the current rate of species extinctions is higher than ever before (Barnosky *et al.* 2011) and this rapid loss of biodiversity threatens our planet. Recently, this fact was not only acknowledged by the scientific community (Naeem, Duffy & Zavaleta 2012; Steffen *et al.* 2015), but also increasingly by policy-makers[1], calling for an immediate halt of biodiversity loss[2] and putting pressure on governments worldwide (Isbell *et al.* 2017b). In the following paragraphs, I will discuss the positive effect of biodiversity on a subset of ecosystem services.

Mitigation of climate change

Biodiversity increases ecosystem temporal stability (Tilman, Reich & Knops 2006; Proulx *et al.* 2010) as well as resistance and resilience towards extreme climatic events such as storms, floods and droughts (Isbell *et al.* 2015), events that are expected to increase in both frequency and severity with climate change (Stocker *et al.* 2013). For a multitude of ecosystems such as forests, meadows, oceans and lakes,

[1] For example the Aichi Biodiversity Targets by the Convention on Biological Diversity (CBD), see https://www.cbd.int/sp/targets/

[2] For example the United Nations Sustainable Development Goals, https://sustainabledevelopment.un.org/sdgs

we know that species diversity can buffer the impact of disturbances imposed by external forces (Balvanera *et al.* 2006; Isbell *et al.* 2015; Duffy *et al.* 2016).

The temporal performance of different species within a community will likely vary if the species possess distinct fundamental niches and life histories (Chesson 2000; Loreau & de Mazancourt 2008). These asynchronous fluctuations among taxa at the population level may result in the maintenance of the overall community performance because the decline in the performance of some species are compensated by other community members such that the overall performance of the community is maintained (Yachi & Loreau 1999; Gonzalez & Loreau 2009; Thibaut & Connolly 2013). Therefore, more diverse communities can enhance the stability of the community because there is a higher probability that some species will maintain the performance of the community within a changing environment, often referred to as the insurance or portfolio effect (Tilman, Lehman & Bristow 1998; Yachi & Loreau 1999; Hector *et al.* 2010; Thibaut & Connolly 2013). At the same time, a larger number of species and higher density can result in stronger competition, increasing the variation in the temporal performance of individual species and thus their temporal asynchrony (Chesson 2000; Loreau & de Mazancourt 2008). Both environmental variation and diversity-competition mechanisms can create asynchronous patterns in the temporal performance of a population that can be quantified and assessed as potential mechanisms behind the stability in the net performance of a community (Thibaut & Connolly 2013; de Mazancourt *et al.* 2013; Gross *et al.* 2014).

Increase of crop yields

Twenty-five years of research, mainly in temperate grasslands, assembled conclusive evidence for the positive effect of plant diversity on productivity (Tilman *et al.* 2001). For agriculture this has important implications, because these findings strongly suggest that biodiversity is a key ingredient in sustainable agroecosystems (Barot *et al.* 2017; Isbell *et al.* 2017a). The diversification of crop cultures is an important approach to maintain high yield (Quijas, Schmid & Balvanera 2010) needed to meet the increasing global food demand (Godfray *et al.* 2010). For agriculture, the diversity of primary producers has a large impact on yields, but in addition, the diversity of plant-interacting species may be equally important (Tooker & Frank 2012). For example, soil microbial diversity has been shown to influence aboveground plant productivity (Wagg *et al.* 2014), knowledge that has been suggested to be used specifically in an agriculture context to increase yields (Bender, Wagg & van der Heijden 2016). In addition, pollinator diversity at least in part increases or maintains plant productivity (Kleijn *et al.* 2015). Biodiversity also increases productivity due to the dilution of species-specific pathogens, both aboveground (Mitchell *et al.* 2003) and belowground (Schnitzer *et al.* 2011a), consequently reducing the prevalence of diseases. Figure 1 summarizes the contrast between monocultures and mixtures.

Increase of other provisioning services

The positive effect of biodiversity was also shown for other ecosystems important for provisioning, such as forests (Paquette & Messier 2011; Gamfeldt *et al.* 2013; Liang *et al.* 2016) and marine ecosystems (Gamfeldt *et al.* 2015; Duffy *et al.* 2016). Straightforward economically valued goods in these systems are timber from commercial production in forests and seafood commercially harvested from fishing in the oceans. In addition, both the oceans and forests are used as hunting grounds and food resource of local people, who rely on local biodiversity and who are often hit the hardest by a loss of the flora and fauna that they depend on for daily survival.

Of course, biodiversity influences a broad range of other ecosystem functions and ecosystem services. This has been reviewed extensively in recent literature (Chapin *et al.* 2000; Hector *et al.* 2001; Loreau *et al.* 2001; Hooper *et al.* 2005; Balvanera *et al.* 2006; Isbell *et al.* 2011; Hooper *et al.* 2012; Reich *et al.* 2012; Cardinale *et al.* 2012; Isbell *et al.* 2017b). For this thesis, I was more interested in extending the work to investigate the mechanisms underlying this positive biodiversity–ecosystem functioning relationship. In particular, I asked: How do short-term evolutionary processes shape the diversity–productivity relationship?

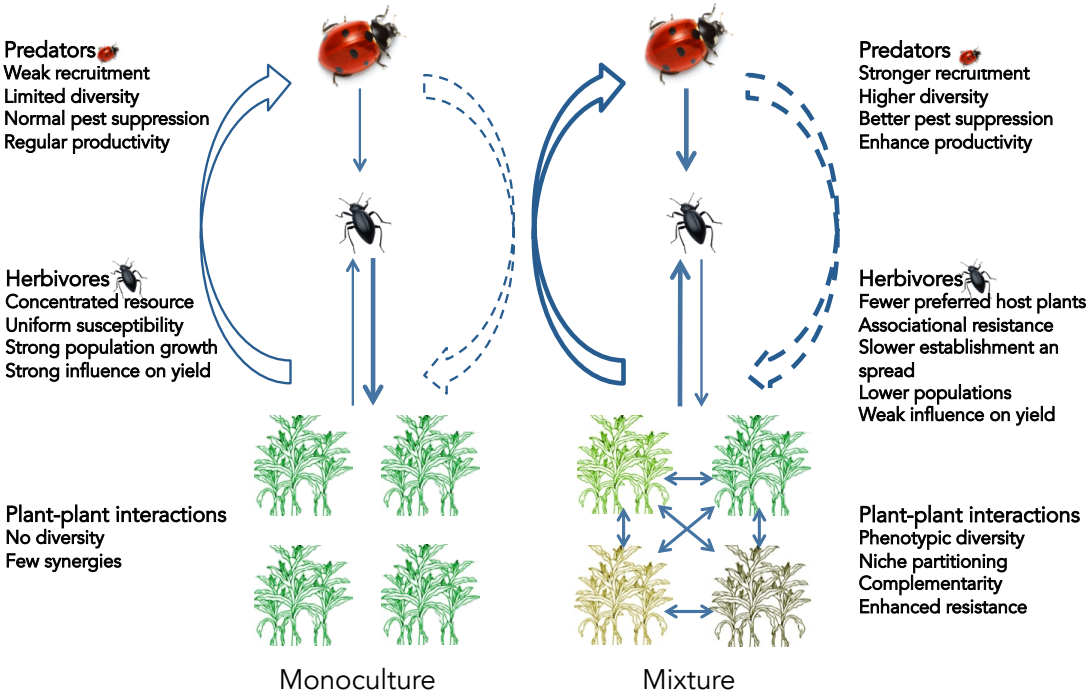

Fig. 1 | Mechanisms of species or genetic diversity improving crop yields. Diversity of plant species influences diversity on several trophic levels and *vice versa*. Interactions between and within these trophic levels determine the outcome, i.e. yields. The width of the arrows indicates the hypothesized relative strengths of the interactions according to literature reviewed in Tooker *et al.* 2012. Illustration adapted from Tooker *et al.* 2012.

Community evolution in grasslands

Time shapes BEF relationships, because with time some members of the community go extinct, new species enter the system, interactions are lost and gained and species adapt to the local conditions. In long-term grassland studies addressing such temporal processes it was found that the positive effect of plant species richness on biomass production increased with time (Cardinale *et al.* 2007; Fargione *et al.* 2007; Reich *et al.* 2012). It is conceivable that functional complementarity between species increases with time. However, only recently evolutionary mechanisms have come to the attention of researchers in plant systems, suggesting that selection of particular genotypes from the total genetic pool of a species may affect ecosystem functioning in field experiments (Zuppinger-Dingley *et al.* 2014b, 2015, 2016b; Kleynhans *et al.* 2016; Rottstock *et al.* 2017). I propose that selection at the level of entire communities is even more likely to affect ecosystem functions, because of non-random niche or trait changes in response to other phenotypes in the community that result in filling niche space more fully or evenly. Hence, I expected that a community of species with a shared selection history would show a stronger biodiversity effect than communities assembled from species without such a shared community selection history. Community evolution was defined as genetically based changes among species in the community resulting in altered species performances and interactions (Whitham *et al.* 2006, explained in detail in the Introduction). But most evidence for community evolution stems from experiments with small organisms with a short generation time, such as bacteria (Fiegna *et al.* 2014). The novel approach of the first two Chapters of this thesis was thus to find evidence for community evolution in perennial grassland species.

Using a long-term biodiversity experiment (the Jena Experiment), I compared selected plant communities with a history of species co-occurrence in the field to naïve plant communities with the same composition but where species lacked a common history of occurrence. For the selected communities, I found a) higher community biomass, b) stronger biodiversity effects, c) higher stability of productivity over time and d) higher resistance towards a flooding event. Taken together, these findings strongly support the notion that evolution within a community can enhance ecosystem functioning. I will now discuss these results in more detail and in a broader context.

Community evolution increases community productivity and stability

In Chapter I found that the difference in yield between species mixtures and monocultures, the so-called biodiversity effect, was larger for plant communities with shared selection history (community evolution) than for plant communities without such shared selection history. This rapid community evolution resulting in increased mixture performance was so far only found for microbial communities, mainly in

laboratory experiments (e.g. Fiegna *et al.* 2014). Here, I found community evolution also for a large number of grassland communities in more natural field conditions (see Chapter 1). The possibility of such evolution for an increased performance of plant mixtures suggests that species loss may be more detrimental than previously thought. Communities cannot be re-assembled by simply adding back populations (or individuals) of the locally extinct species, because these populations lack a common selection history with the rest of the community.

In Chapter 2, I used the same experimental framework as in Chapter 1; however, I focused on the temporal variation of productivity during the course of the experiment. The diversity–stability relationship has received great attention over the past decades and it was shown that biodiversity increased stability in grassland plant communities during unperturbed states and in response to extreme weather events such as droughts and floods (Tilman *et al.* 1994; Proulx *et al.* 2010; Isbell *et al.* 2015). However, the influence of community evolution on stability has to the best of my knowledge never been studied. We hypothesized that not only plant diversity can buffer plant communities, but that also community evolution may increase ecosystem stability both during perturbed and unperturbed states. Using data from seven harvests I observed that selected plant communities showed higher ecosystem temporal stability over four years compared to naïve plant communities. In addition, these selected communities recovered better from a flooding event in spring 2013 (see Chapter 2).

Extreme weather events such as storms, droughts or floods are increasing in both frequency and severity with climate change, urging ecosystems to cope with these perturbations. Maintaining the resilience of ecosystems is crucial for conservation purposes and ecosystem management (Scheffer *et al.* 2001), which makes unraveling the mechanisms increasing stability so important. Here, I report for the first time that community evolution can increase stability and recovery of such ecosystems.

Chapters 1 and 2 establish the importance of community evolution for the functioning of grassland ecosystems. Harper (1962) already recognized the importance of studying plants within communities and not in isolation and wrote: "The form, tolerances and persistence of species may be profoundly modified by the proximity of neighbours of the same or other species. It follows that the characteristics of individual species shown by isolated individuals or pure populations may offer no significant guidance to their behavior in the presence of others. Conversely, the ecology and distribution of a species in the presence of others may offer no significant guide to the behavior of isolated individuals. […] Individuals free from the influence of neighbours are anomalies in nature. […] As the behaviour of individuals is modified by their neighbours, so the population acquires its own distinctive physiology – different from that of isolated plants."

This statement does not only hold true for experimental approaches and designs, but more importantly, can be extended to conservation practices. I conclude that to protect species performances and interactions, conversation strategists should

increase their efforts to preserve entire communities, which is essential for maintaining ecosystem functions and services and the resilience of natural ecosystems to extreme events.

Community diversity acts as selective pressure leading to differentiation into monoculture and mixture sub-types within a species

In Chapter 3, I took a contrasting approach than in Chapters 1 and 2. Here I studied diversity as selective force *within* selected plants. I would like to emphasize the fundamental differences between these two approaches. Whereas in Chapters 1 and 2, I investigated the role of community evolution on entire plant communities, in Chapter 3 I asked whether community diversity could lead to selection at the level of single species. In particular, I tested whether community diversity can act as selective force that differentiates plant populations of a given species into mixture and monoculture sub-types.

Intra- and interspecific competition result in different selection pressures on plant individuals in communities of differing species diversity. These differences between monoculture and multispecies communities in turn lead to differential evolution for monoculture and mixture sub-types within a plant species. Indeed, an earlier study found that experimental mixtures consisting of mixture-type plants outperformed those consisting of monoculture-type plants, and, vice versa, that monoculture-type plants outperformed mixture-type plants in newly assembled test monocultures (Zuppinger-Dingley *et al.* 2014b). Furthermore, it was shown that these sub-types differed in their metabolic fingerprint (Zuppinger-Dingley *et al.* 2015). Based on these results, I conducted a glasshouse pot experiment testing not only for differentiation into monoculture vs. mixture types, but also comparing these two to naïve plants lacking any selection history in the field. I compared the growth performance of plants with a 12-year selection history in mixed cultures or monocultures and of naïve plants without a selection history in the experiment during 24 weeks of growth in the greenhouse.

Plants without a past community selection history produced the lowest community biomass and showed the weakest biodiversity effects. Furthermore, I found that twelve years of selection history in monocultures or species mixtures differentiated plants into monoculture- and mixture-types within species. In newly assembled mixtures, plants with a selection history in mixtures performed better than plants with a monoculture selection history. Biodiversity effects were generally positive, but contrary to expectation, not stronger for mixture types. In addition, biodiversity effects were both influenced by trait differences among plants and community-weighted means, but these relationships were largely independent of selection history.

The influence of the selection history on biodiversity effects suggests that evolutionary processes can shape the biodiversity–ecosystem functioning relationship

already within a few generations. Thus, I believe that this study may provide an explanation for the strengthening of the biodiversity–ecosystem functioning relationship over time, which has been observed in biodiversity experiments in Cedar Creek, USA and Jena, Germany. I conclude that revealing such rapid evolutionary processes in grassland plant communities also has implications for conservation strategies. Thus, it may not be sufficient to only conserve species in isolation but rather in communities or populations of species with co-evolved interactions.

Epigenetic and genetic variation between populations of grassland species

For this dissertation, I was interested in short-term evolutionary processes within species, occurring over only few generations, as opposed to long-term evolution in the context of speciation. This rapid evolution has been defined "as a genetic change occurring rapidly enough to have a measurable impact on simultaneous ecological change" (Hairston *et al.* 2005). Genetic variation is a prerequisite for rapid evolution and the basis for speciation, adaptation and extinction. Several processes can lead to adaptation. For organisms with fast generation times and asexual reproduction, such as clonal populations of bacteria, mutations (Tenaillon *et al.* 2016) and horizontal gene transfer (Soucy, Huang & Gogarten 2015) are the main sources of adaptation. However, for species with longer generation times, selection far more often acts on standing genetic variation (Barrett & Schluter 2008), resulting in a sorting-out of suitable genotypes (Fakheran *et al.* 2010). Hence, rapid evolution can also occur in species with a long generation time, as was shown for a vertebrate, i.e. the killifish (Reid *et al.* 2016) and for plants such as *Brassica rapa* (Gervasi & Schiestl 2017), *Mimulus gattus* (Bodbyl Roels & Kelly 2011) and several common grassland species (Zuppinger-Dingley et al. 2014).

In a glasshouse experiment we observed stronger biodiversity effects for plant communities consisting of plants selected in plant mixtures, compared to monocultures (see Chapter 3). In Chapter 4 I then aimed to find the mechanisms underlying this phenotypic variation observed in the glasshouse. Due to the common garden nature of the experiment, phenotypic plasticity could be ruled out as a mechanism and a genetic signal was to be expected.

However, it was also proposed that epigenetic adaptation was driving our results described in Chapter 3 and earlier findings by Zuppinger-Dingley et al (2014). In a comment accompanying the latter, David Tilman and Emilie Snell-Rood wrote: "In Zuppinger-Dingley and colleagues' study, laboratory propagation of the plants increased the chance that the differences between the high- and low-diversity selection groups were due to genetic divergence. However, it is possible that epigenetic factors — heritable changes that do not involve DNA-sequence changes — could have had a simultaneous role" (Tilman & Snell-Rood 2014). Epigenetic variation describes meiotically heritable changes in gene expression without changes to the underlying DNA sequence (Verhoeven *et al.* 2016). Therefore, we analyzed

plant material from plant individuals from three selection histories: selection history "mixture", "monoculture" and "none" using a novel reference-free epiGBS method (van Gurp *et al.* 2016), which includes genetic and epigenetic analysis of plant material.

I analyzed genetic and epigenetic variation between populations of six common grassland species. Specifically, I tested for genetic and epigenetic variation in mixture and monoculture sub-types within one species. Previously it had been shown that these sub-types differed in their metabolic fingerprint (Zuppinger-Dingley *et al.* 2015), hence it was likely that they differed also in their "genetic fingerprint". EpiGBS output classified plants within a species as either monoculture- or mixture-selection history based on their single nucleotide polymorphisms (SNP) in a representative part of the genome. Epigenetic variation between individuals of the same species was the result of underlying genetic variation.

Our results indicate that, in perennial grassland species, it may be a genetic signal that drives the rapid emergence of monoculture and mixture sub-types. I propose that pre-adapted genotypes or epigenetic variants were sorted out from the standing genetic or epigenetic variation (Bossdorf et al. 2008).

In Chapter 4 I conducted genetic analyses on non-model grassland species. My study is the first of its kind trying to pin down the mechanism behind putative rapid evolution in grasslands. My findings suggest that selection on standing genetic variation seems to be a powerful driver of evolution even in the absence of many generations of plant growth. In addition, I propose that community diversity had the selective power to differentiate plant populations within species into mixture and monoculture sub-types within only a few years. I conclude that molecular tools and the integration of evolutionary concepts into plant community ecology can open up a new alley of exciting research, which should be exploited to understand the community evolutive processes that lead to the plant community compositions and structures as we see them today.

Plant–soil feedbacks as a driver of positive biodiversity effects in grasslands

The fifth Chapter of this thesis explored the role of positive plant–soil feedbacks for the strengthening relationship between biodiversity and ecosystem functioning. Such positive plant–soil feedbacks have often been attributed to arbuscular mycorrhizal fungi (AMF), ubiquitous soil-borne fungi able to form symbiotic relationships with plants (van der Putten *et al.* 2013a). By penetrating a host's root parenchyma, the fungus extracts plant-derived carbohydrates (Smith & Smith 2011) and in exchange provides mineral nutrients to the host (Gianinazzi-Pearson 1996; van der Heijden *et al.* 2006). AMF have the potential to improve plant survival and growth at certain conditions by increasing nutrient uptake of the host plant (Jones & Smith 2004; van der Heijden *et al.* 2006). Associations between plants and plant-beneficial or -detrimental soil organisms have received much attention in

the past decades (e.g. Bever 1994; Klironomos 2002; van der Heijden, Bardgett & van Straalen 2008; Kardol *et al.* 2013; van der Putten *et al.* 2013) but often studies have not considered that interactions between plants and such organisms may change over ecological time-scales through adaptation (Lekberg & Koide 2014). In particular, the dependence of co-adaptation of plants and beneficial soil organisms to local plant diversity has never been tested experimentally. However, it has been shown that plants with a selective past in monocultures can evolve positive plant–soil feedbacks after eight years of growing in a long-term biodiversity experiment in Germany (the Jena Experiment, Zuppinger-Dingley *et al.* 2016).

In Chapter 5 of this thesis, Terhi Hahl was the leading researcher. She tested these plants with a selective past in monocultures and compared them with plants of the same species but with a selective past in mixtures. We had hypothesized that either co-adaptation with AMF or increased pathogen defence could have resulted in the increased positive plant–soil feedbacks for monoculture plants which had been observed previously (Zuppinger-Dingley *et al.* 2016b). We conducted a glasshouse experiment using seven grassland plant species selected in monocultures (monoculture-type plants) or mixtures (mixture-type plants).

Hahl *et al.* found mixed evidence for co-adaptation between monoculture-type plants and monoculture AMF and between mixture-type plants and mixture AMF. Interestingly, in most cases the co-adaptation was detrimental rather than beneficial for the plants, indicating a delicate balance where co-adaptation can increase mutualism or parasitism of the specific plant–AMF interaction. Monoculture-type plants suffered less damage from aboveground pests in the glasshouse, but for most species this came at the cost of reduced growth compared with mixture-type plants.

With this I conclude the discussion of the chapter-specific results and will now expand on some results I obtained from trait measurements both in the glasshouse and in the field.

The importance of intra- and interspecific trait variation for species co-existence

Traditionally, between-species trait variation was used to explain coexistence and niche differentiation between species. A set of traits is measured in a number of species and trait means subsequently used for each species individually to compare between-species trait variation (Albert *et al.* 2010). This same method can then be expanded to comparing trait means between populations of the same species, but in varying diversity levels. For example, Gubsch *et al.* (2011) observed that species diversity increased specific leaf area (SLA) in several grass species. Similarly, Roscher *et al.* (2011) observed that SLA was higher in more diverse communities for twelve legume species. Such interspecific trait variation between species is especially important when two closely related species have overlapping ranges. In this situation, character displacement (Brown & Wilson 1956) can enable coexistence. Where one

species occurs alone, the populations of that species are similar to the other species and difficult to distinguish from each other. In contrast, where the two species occur together, the populations are more divergent and easily distinguished, i.e., they 'displace' one another in one or more characters. The characters involved can be morphological, ecological, behavioral, or physiological; they are assumed to be genetically based (Brown & Wilson 1956).

More recently, it has also been acknowledged that *intra*specific trait variation might play a similarly important role (Bolnick *et al.* 2011; Albert *et al.* 2011; Violle *et al.* 2012). In my first Chapter, I showed that populations of the same species differed in their within-species trait variation for SLA. Depending on their selection history, variation in SLA differed in low- and high-diversity plots. In other words, plants with a community selection history showed higher within-species variation in SLA in low diversity, whereas naïve plant communities showed higher intraspecific variation in high diversity. The narrowing of within-species variance with increasing diversity in selected communities may thus underlie character displacement between species (Brown & Wilson 1956). In contrast, species in monocultures may have been selected for niche expansion resulting in increased within-species variance. Species in naïve communities had not yet responded to different diversity treatments with a similar adjustment in within-species variance in the four years of this study; their higher variance at high diversity may stem from a more heterogeneous biotic environment.

In my third Chapter, I addressed the importance of both inter- and intraspecific trait variation in a pot experiment. In 2-species and monoculture test communities I measured several traits of the four plant individuals growing in each pot. This allowed me to quantify both inter- and intraspecific variation for plant height, SLA and leaf thickness in both monoculture vs. mixture test assemblies. Then I compared test communities consisting of plants with a history of growing in mixtures vs. test communities consisting of plants with a selection history of growing in monocultures.

I hypothesized that mixture-type plants should exhibit larger trait variation between species as they underwent selection for increased complementarity during twelve years in the experimental field plots. Conversely, I expected stronger within-species trait variation in monoculture-type plants, due to twelve years of strong intraspecific competition in the experimental field plots. To my surprise, I found that variation tended to be larger both within and between species for monoculture-type plants, thus not confirming my hypotheses. Several studies have investigated the relationship between species richness and community-level trait variation (Hulshof et al. 2013, Le Bagousse-Pinguet et al. 2014, Lamanna et al. 2014, Siefert et al. 2015) and found that the relative extent of intraspecific trait variation depended on species richness. In monocultures, a large intraspecific variation is advantageous for a more efficient resource use. Thus, the observed trend for increased trait variation in monoculture types is consistent with potential selection for within-species niche differentiation and character displacement between genotypes in monocultures.

My results for mixture-type plants contrast with the findings of an earlier study in which increased complementarity effects were associated with increased

between-species trait differences (Zuppinger et al. 2014). A potential explanation for the contrasting results is that the earlier study used species more dissimilar from each other, namely grasses, legumes, small herbs and tall herbs. Species in the present study were more similar, behaving more as a monoculture and therefore perhaps less likely to further increase their differences by short-term evolution than species being more different to begin with and behaving more as a true mixture. The mixture-type plants of the different species in the present study may have evolved "parallel" character displacement in response to species of the other functional groups also present in the mixtures in which they were selected in the Jena Experiment.

Perspectives and future research

I made the novel finding that selection in grasslands can occur much faster than previously thought, which has large implications on how we view the biodiversity–ecosystem functioning relationship. Evolutionary approaches will be needed for future research in community ecology trying to unravel the mechanisms underlying the increase of biodiversity effects over time (Reich *et al.* 2012). With current rapid advances in sequencing technology, such approaches will be more economical and feasible. Working with non-model species is of course challenging, but in my opinion the field of community ecology can only advance by integrating both, the methods and also importantly the theory of evolutionary biology.

I would like to conclude this thesis by emphasizing once more the importance of biodiversity for humanity. Humans continue to alter the global environment, decreasing biodiversity and as a consequence, jeopardizing ecosystems functions that are crucial to our survival. Current extinction rates per [time] are a 100 to 1'000 times greater than pre-human rates and unrivalled by anything this planet has experienced in the past (Pimm *et al.* 1995; Barnosky *et al.* 2011). In addition to these species extinctions, a severe population decline, in particular in vertebrate species, is fueling a "biological annihilation" of our planet (Ceballos, Ehrlich & Dirzo 2017). In the absence of policy changes, this trend will continue and may have devastating impacts on our Earths inhabitants, plants, animals and humans alike (Chapin *et al.* 2000). This should motivate scientists all over the world to continue to research the importance of biodiversity for ecosystem functions and communicate our findings to the general public. Only then will we be able to meet Aichi Biodiversity Target Number One[3]: "By 2020, at the latest, people are aware of the values of biodiversity and the steps they can take to conserve and use it sustainably."

[3]https://www.cbd.int/sp/targets/

BIBLIOGRAPHY

Correlation is innocent until proven guilty.

- George Sugihara

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ACKNOWLEDGMENTS

It is our choices… that show what we truly are, far more than our abilities.

- Prof. Dumbledore in Harry Potter and the Chamber of Secrets (1998)

Thanks to

Bernhard Schmid for giving me the opportunity to conduct my PhD independently and for providing me with many statistical and linguistic tools that will be indispensable for my future career. Your ideas and enthusiasm will surely resonate for many, many years to come. I am honored that I had the opportunity to work with you and to be one of your last students to finish. I hope I could make you at least a little bit proud.

Terhi Hahl for sharing the entire PhD with me. You were always there and were the brain of the whole data collection special operation. Thanks, Terhi, you are amazing.

the committee members, Kentaro Shimizu, Mark van Kleunen and Marcel van der Heijden for your support and valuable input during the committee meetings.

my collaborators in Holland, Philippine Vergeer, Niels Wagemaker and Thomas van Gurp, for your bravery to work with me and for being fantastic hosts in Nijmegen. It was such a pleasure, van harte bedankt!

Cameron Wagg for helping with data analysis whenever I was stuck. Your passion and dedication for science are inspiring and I am glad we shared this time together. Special thanks for career advice and your friendship.

Marc Schmid for arriving at the perfect time to help me writing papers, analyzing data and finishing my PhD. You were always there when I had R troubles and your cleverness motivated me to always give my best.

Matthias Furler, Dorde Topalovic and Theres Zwimpfer for your help with glasshouse and garden matters, René Husi for your help in the lab and Isabel Schöchli, Lilian Dutoit, Jacqui Moser and Michel Nakano for your administrative support.

Daniel Trujillo for being the best slave and friend anyone could wish for. One day we will go back to Jena again and enjoy a delicious German meal. It will be great.

Helmi Brandl for your enthusiasm for science, for introducing me to science outreach and for your support. I hope you are in a wonderful place now, surrounded by microbial life. You are greatly missed.

the many helpers in the field, especially to Abdulmaged Alhemiary and Carsten Möller (a.k.a. Carpra) and the helpers in the glasshouse, Nadia Castro, Enrica de Luca, Matteo Brezzi and Patrick Gyöngy, who worked very hard, and miraculously survived my reign of terror. You are strong people!

my office mates and neighbor-office mates (only a thin wall separated us after all…): Yuanyuan Huang, Maitane Iturrate, Elvira Schnyder, Jacqueline Oehri, Gianalberto Losapio, Nina Gerber and Anaïs Tilquin. I am grateful for your helpful comments on about everything I needed feedback on (presentations, interviews, General Introduction, which menu is best in the Mensa…)

Elisa T. Granato who inspired me to do this PhD in the first place and who continues to be a motivating and enthusiastic colleague and friend. #leanin

Samuel Wüest for your support, enthusiasm, career advice and positive energy. Your radiant smile has saved many a day!

Pascal Niklaus for help with anything that had to do with soil and for solving R troubles and letting me join your retreat.

Debra Zuppinger-Dingley for the help with everything from setting-up the experiment, to data analysis, to manuscript writing and for being such a good friend.

the amazingly talented two Zumba teachers Adele Ferrari and Janielle Porter for providing such a great way to relax and party in the evening. A very motivating sportive activity and ideal for a stressed-out PhD candidate!

all my friends at the University of Zurich for making my PhD a great time, for the many good hours on the glasshouse roof or at the tomato place and for your friendship.

Last but not least, I want to thank my family. Thanks to

my husband Tobias Vogler, for your unconditional love and support and a lot of enthusiasm for my work and nature in general.

my son Liev for being such a relaxed, happy and brave boy and for waking me up every morning at 7 am.

my parents for supporting me throughout my academic career and my parents-in-law for help and support.

all my extended family for your constant support and enthusiasm for my work.

It needs a village to finish a PhD!

CURRICULUM VITAE

Lean in.

- Sheryl Sandberg

Personal

Surname	VAN MOORSEL
First names	Sofia Julia
Date of Birth	9.6.1987
Place of origin	Zürich, ZH

Education

2013 – 2017	PhD student at the Life Science Zurich Graduate School PhD Program: Plant Sciences Advisor: Prof. Bernhard Schmid, Department of Evolutionary Biology and Environmental Studies, University of Zurich
2012 – 2013	MSc Biology/Microbiology, University of Zurich Title of thesis: High-throughput assessment of metabolic pathways in *E. coli*. Supervisors: Prof. Dr. Martin Ackermann and Dr. David Johnson, EAWAG, Switzerland
2008 – 2012	BSc Biology, University of Zurich
2007 – 2008	Two semesters studies of multilingual communication, Zurich University of Applied Sciences
2002 – 2006	Eidgenössische Matura, Neusprachliches Profil (Schwerpunkt Spanisch), Kantonsschule Zürcher Oberland, Wetzikon

Publications

van Moorsel, S.J., Hahl, T., Wagg, C., De Deyn, G.B., Flynn, D.F.B., Zuppinger-Dingley, D. and Schmid, B. Community evolution increases plant productivity at low diversity (*in revision for Ecology Letters*)

van Moorsel, S.J., Schmid, M.W., Hahl, T., Zuppinger-Dingley, D. and Schmid, B. Selection in response to community diversity alters plant performance in newly assembled test communities (*under review at Ecology*)

Svercel, M., Saladin, B., **van Moorsel, S.J.**, Wolf, Sarah & Bagheri, H.C. Antagonistic interactions between filamentous heterotrophs and the cyanobacterium *Nostoc muscorum*. *BMC Research Notes* **4:**357 (2011)

Conferences and research seminars

9/2017	40th New Phytologist Symposium, Vienna, AT *Selected oral presentation and poster presentation*
3/2017	Aquatic ecology & macroevolution seminar series, EAWAG Kastanienbaum, CH *Oral presentation*, *invited speaker*
3/2017	Institute of Plant Sciences, University of Bern, CH *Oral presentation, invited speaker*
2/2017	Biology17, Bern, CH *Oral presentation*
12/2016	British Society of Ecology (BES) annual meeting, Liverpool, *Oral presentation*
11/2016	BEEES Seminar at the University of Zurich, CH *Oral presentation*
8/2016	Global Change and Biodiversity Conference, Monte Verità, CH *Poster presentation*
8/2016	Ecological Society of America (ESA) annual meeting, Fort Lauderdale, USA *Oral presentation*
2/2016	Biology16, Lausanne, CH *Poster presentation*
2/2016	Symposium of the Zurich-Basel Plant Science Center, Zurich, CH *Poster presentation*
8/2015	100th Ecological Society of America (ESA) annual meeting, Baltimore, USA *Oral presentation*
6/2015	epiGBS Symposium at the Radboud University, Nijmegen, NL *Oral presentation, invited speaker*
5/2015	28th Plant Population Biology Conference, Tubingen, DE *Oral presentation*
5/2014	27th Plant Population Biology Conference, Konstanz, DE *Poster presentation*
5/2014	33rd New Phytologist Symposium, Zurich, CH *Poster presentation*

Awards and Grants

7/2017	New Phytologist Trust travel grant (620 Euros)
2/2017	Presentation award at the Biology17 conference in Bern (CH)
8/2016	Poster award at the Global Change and Biodiversity Conference in Monte Verità (CH)
6/2015	Short visit travel grant from the ESF Congenomics Network (1800 Euro)
5/2014	Poster award at the PopBio Conference in Konstanz (DE)

APPENDIX

Award winning poster for the PopBio Conference in Konstanz, Germany (May 2014)

Evolution of monoculture and mixture types in a grassland biodiversity experiment

Sofia van Moorsel*, Debra Zuppinger-Dingley, Terhi Hahl and Bernhard Schmid

University of Zurich, Institute of Evolutionary Biology and Environmental Studies

BACKGROUND

Ecosystem functioning generally increases with increasing biodiversity.

Communities with high species diversity are more productive than such with lower diversity. **This positive effect of biodiversity generally increases over time.** Recent studies have shown grassland plants surviving for eight years in mixed species communities have been selected for better performance in mixed communities (mixture types) in contrast to plants selected in monocultures (monoculture types), and vice versa (Figure 1). Additionally, **mixture types demonstrated stronger biodiversity effects than monoculture types.**

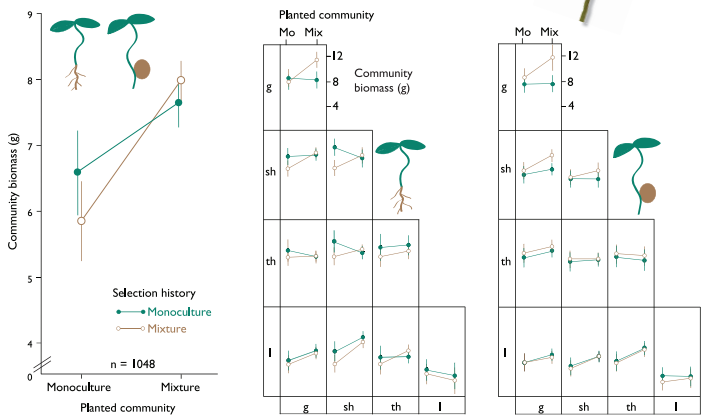

Figure 1. (A) Plants selected over 8 years in mixtures produced greater biomass in new test mixtures in contrast to plants selected in monocultures, as indicated by the difference in slopes. Such an **increase in productivity for mixture types planted in test mixtures** appeared to be a general trend for both (B) cuttings and (C) seedlings and functional group combinations. The plots are ordered to functional group combinations: grasses (g), short herbs (sh), tall herbs (th), legumes (l).

IMPLICATIONS

Different selection pressures on plants occurring within monocultures or mixtures may result in **selection for monoculture and mixture genotypes** respectively within such communities. Mixture types performed better in species mixtures due to an **increase in complementarity effects** via selection for trait divergence between species in mixtures (data not shown).

FUTURE EXPERIMENTS

I will set up a series of experiments to test for:

1. **selection for increased combining ability** in mixtures, the ability for increased complementarity between species of mixture types in contrast to monoculture types, and
2. **genotypic differences** between monoculture and mixture types.

OPEN QUESTIONS

What makes monocultures plants perform better in monocultures?

Have they been selected for better defenses against pathogens?

Acknowledgments

SNF grant for funding, Tobi Vogler, Theres Zwimpfer, Daniel Trujillo

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University of Zurich[UZH]

FNSNF

Poster for the PopBio Conference in Lausanne, Switzerland and for the Plant Science Center Symposium in Zurich, Switzerland (February 2016)

Is there evidence for rapid evolution in a long-term grassland biodiversity experiment?

Sofia van Moorsel*[1], Terhi Hahl[1], Debra Zuppinger-Dingley[1], Philippine Vergeer[2], Joop Ouborg[3] and Bernhard Schmid[1]

[1]Department of Evolutionary Biology and Environmental Studies, University of Zurich, Switzerland
[2]Wageningen University, the Netherlands
[3]Radboud Unversity Nijmegen, the Netherlands

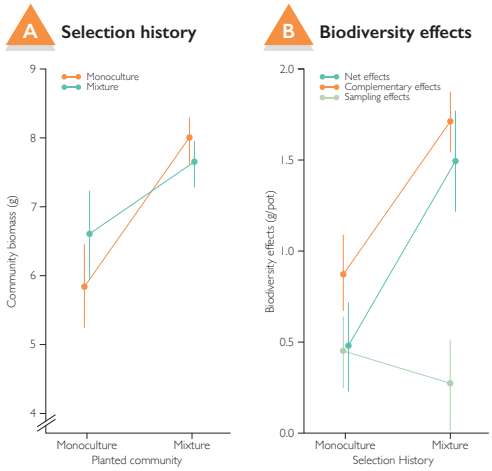
BACKGROUND

Plant communities with high species diversity are more productive than those with lower diversity. **This positive effect of biodiversity generally increases over time.**

Recent studies have shown grassland plants surviving for eight years in mixed species communities have been selected for better performance in mixed communities (mixture types) in contrast to plants selected in monocultures (monoculture types), and vice versa (Figure 1A).

Additionally, mixture types demonstrated stronger biodiversity effects than monoculture types (Figure 1B). For the first time we have evidence for **rapid evolution in grassland ecosystems.**

PREVIOUS RESULTS



◀ Figure 1.
A Plants selected over eight years in mixtures produced greater biomass in new test mixtures in contrast to plants selected in monocultures, as indicated by the difference in slopes.
This **stronger biodiversity effect in mixture types** appeared to be a general trend for both cuttings and seedlings and functional group combinations.

B Mixture types performed better in species mixtures due to an **increase in complementarity effects** via selection for trait divergence between species in mixtures.

(Zuppinger - Dingley et al., 2014 in *Nature*)

EXPERIMENT

In a glasshouse experiment (see photos) we tested:

1. **Selection for increased combining ability** in mixtures, and for increased complementarity between species of mixture types and monoculture types.

2. **Genetic and epigenetic differences** between monoculture and mixture types of the same species.

I assembled two-species mixtures and monocultures of nine European grassland species in pots each with four competing plants. After 20 weeks of growth, I harvested the aboveground biomass of all the individuals and measured phenotypic traits in order to assess their complementarity. The leaf samples for the subsequent genetic and epigenetic analysis were collected after 10 weeks of growth and frozen at -80° C.

OUTLOOK

Samples from monoculture and mixture type plants are currently being sequenced in order to determine differences in **methylation patterns and SNP's.**

To test for genetic and epigenetic differences we make use of a reduced representation bisulfite sequencing (RBBS) technique that enables us to screen both genetic and epigenetic variation in a cost-efficient and highly detailed way. We expect first results in the beginning of 2016.

Acknowledgments
SNF grant for funding, Tobias Vogler, Daniel Trujillo, Matthias Furler, Dorde Topalovic, Niels Wagemaker, URPP GCB, ESF Congenomics Network

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FNSNF Swiss National Science Foundation

University of Zurich[UZH]

Award winning poster for the Global Change and Biodiversity Conference in Monte Verità, Switzerland (August 2016)

Evidence for Rapid Evolution in Grasslands

Sofia van Moorsel[1], Niels Wagemaker[2], Philippine Vergeer[3], Terhi Hahl[1], Debra Zuppinger-Dingley[1] and Bernhard Schmid[1]

[1]Department of Evolutionary Biology and Environmental Sciences, University of Zurich, Switzerland
[2]Department of Experimental Plant Ecology, Radboud University, Nijmegen, the Netherlands
[3]Department of Environmental Sciences, Wageningen University, the Netherlands

We tested whether **selection for monoculture and mixture types** occured at an **epigenetic and/or genetic** level in nine plant species growing in mixtures and monocultures for 12 years.

Our results show selection for monoculture and mixture types ocurred. **Plants with a selection history in mixtures performed better** than plants with a selection history in monoculture (A) and showed higher between species variation (B) in newly assembled mixtures.

Plants within species could be classified from either monoculture or mixture selection history based on their methylation pattern and single nucleotide polymorphisms using epiGBS (van Gurp et al., 2015). (C)

We found evidence for rapid evolution in grassland species at both a genetic and epigenetic level!

The next step is to examine which driver is stronger and how these changes relate to phenotypic differences within species.

Want to know more? Please get in touch!

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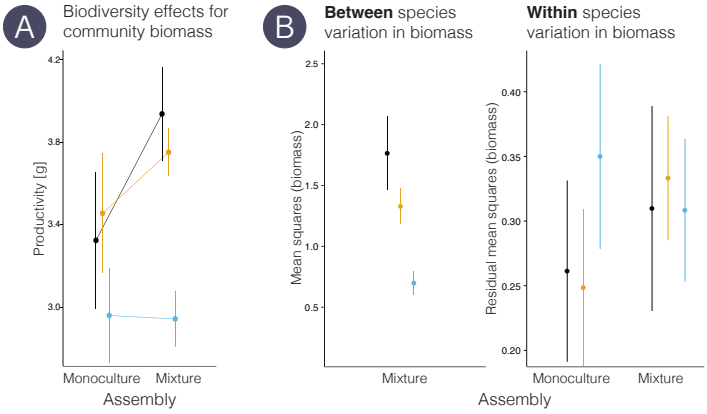

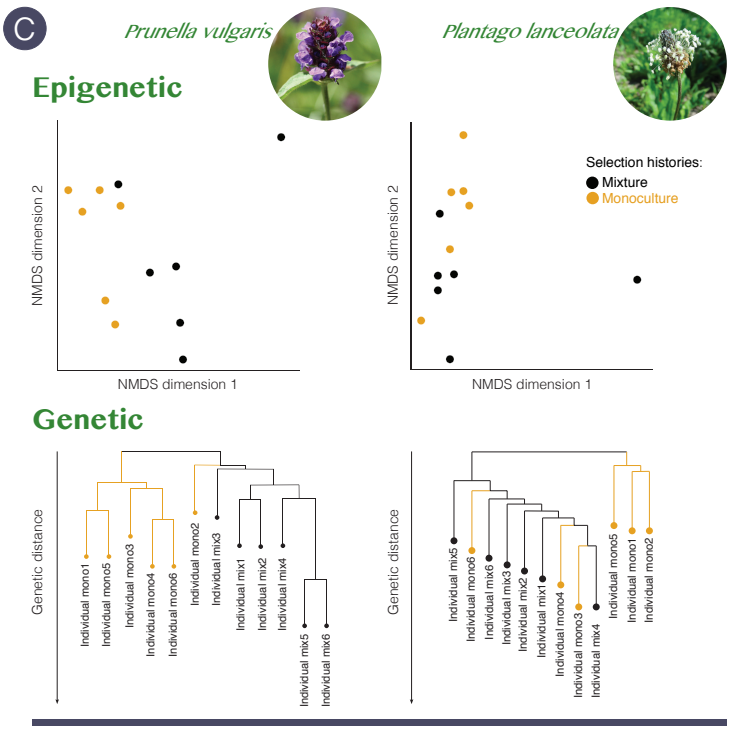

We would like to thank the SNF for funding and the ESF Congenomics Network for a travel grant. The bioinformatics was performed by Thomas van Gurp. A big thanks goes to D. Trujillo, M. Furler and D. Topalovic for technical assistance in the greenhouse and field. We are also grateful to N. Castro. E. De Luca, M. Brezzi and T. Vogler for their help and the URPP GCB network for scientific support.